ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

FORMULATION AND OPTIMIZATION OF TASTE-MASKED ORALLY DISINTEGRATING PHENOBARBITONE TABLETS PREPARED BY DIRECT COMPRESSION METHOD

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May 2015
ADDIS ABABA

FORMULATION AND OPTIMIZATION OF TASTEMASKED PHENOBARBITONE ORALLY DISINTEGRATING TABLETS PREPARED BY DIRECT COMPRESSION METHOD

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A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements of the Degree of Master of Science in Pharmaceutics in the Department of Pharmaceutics

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This is to certify that the thesis prepared by Wondesen Gadisa, entitled: *Formulation and Optimization of Taste-Masked Phenobarbitone Orally Disintegrating Tablets Prepared by Direct Compression Method* and submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmaceutics complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Place and date of submission: Addis Ababa, Ethiopia, May. 2015

ACKNOWLEDGEMENTS

I offer my adoration to Almighty God who gave me the strength and courage to complete my thesis, and the opportunity to thank all those people who contribute their unreserved support for the completion of the work.

I take it as a privilege to sincerely express my deep sense of gratitude and thank my advisors, Dr. Anteneh Belete and Dr. Fitsum Feleke Sahle whose meticulous guidance, valuable suggestions, constant motivation and dynamic approach towards students built my confidence during the course of study and enabled me to complete the thesis work.

I extend my heartfelt thanks to Addis Pharmaceutical Manufacturing Sh.Co. (APF) for donating Phenobarbitone raw material and Eudragit® E100; Food, Medicine and Health care Administration and Control Authority of Ethiopia for authorization of phenobarbitone. Ethiopian Pharmaceutical Manufacturing Sh. Co. (EPHARM) for providing me access to their laboratory for the various studies.

I would like to acknowledge Adama Science and Technology University for sponsoring my postgraduate study and Addis Ababa University for sponsoring my thesis research.

I extend my heartfelt gratitude to Ato Fekade Tefera for his technical support in using laboratory facilities.

I thank all the teaching and support staff of the School of Pharmacy for their help during my study in providing the necessary materials.

I would also like to thank all my family, friends and others who were directly or indirectly involved in motivating, encouraging and supporting me all throughout my career

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ABSTRACT

Epilepsy is a serious neurological disorder characterized by spontaneous seizures. Despite the development of successive generations of antiepileptic drugs, phenobarbitone has retained a unique position in the therapeutic armamentarium and is still the most widely prescribed drug for treatment of epilepsy throughout the world. However, the pharmaceutical industry supplies oral solid dosage forms that are generally inadequate for pediatric, geriatrics and patients experiencing difficulty in swallowing needs. Therefore, the present study aims at formulation and optimization of taste-masked orally disintegrating tablets of phenobarbitone using direct compression technique for use in specific population such as pediatrics, geriatrics and dysphagia patients. Thus, the study begins with the determination of the bitterness threshold of phenobarbitone *in vivo*. Then, taste-masked microspheres of phenobarbitone were prepared by oil in water emulsion solvent evaporation technique using Eudragit®E100 as a polymeric material.

The effect of formulation variable i.e., polymer: drug ratio at four different levels (1:1, 2:1, 3:1 and 4:1) and process variable, i.e., stirring rate (500, 650 and 800 rpm) were examined. The prepared formulations were characterized for flow properties, particle size distribution, entrapment efficiency and percentage yield. The study revealed that the mean particle size ranged from 386.01 ± 3.88 to 456.72 ± 3.53 μm, the drug encapsulation efficiency varied from 88.50 ± 2.14 to 97.00 ± 1.26% of the theoretical amount incorporated. The taste-masking efficiency was determined by spectrophotometric analysis based on the amount of drug released from taste-masked microspheres in pH 6.8 phosphate buffer after 5 min. Taste evaluation studies confirmed that microspheres of phenobarbitone having a polymer: drug ratio of 1:1 are tasteless. FT-IR spectra of phenobarbitone and the physical mixture of drug and Eudragit®E100 1:1 suggested no interaction between the drug and polymer.

The effect of various formulation and process variable on phenobarbitone orally disintegrating tablets was investigated. The results of the experiments revealed that the major factors that affect the tablet characteristics are compression force, level of superdisintegrant and MCC/mannitol. Besides, since it has significant effect on drug release, the effect of polymer to drug ratio within the microsphere on the various tablet characteristics was also investigated. Thus, four factors, two level (2^{4-1}) fractional factorial experimental design was selected to investigate the effects of the selected independent variables on the various responses such as disintegration time, wetting time, hardness, drug release in 5 min and drug release in 15 min of taste-masked orally disintegrating tablets (ODTs). Accordingly, the various models describing the relationship of the selected variables were obtained using Design-Expert 8.0.7.1 software and the models were analyzed. The corresponding surface response and contour plots were also obtained and the optimum area was determined and, finally, the optimum was validated.

Simultaneous optimization of the responses gave the most desirable representative optimum formulation, within the common optimum region, with a disintegration time of 31.95 sec., wetting time of 46.41 sec., hardness of 52.13 N, drug release within 5 min 62.21%,and drug release within 15 min of 90.03% at compression force 11.29 KN, SSG of 4.6%, MCC/ mannitol ratio of 1.6 :1 and polymer to drug ratio of 1.26:1. The validity of obtained optimal point was confirmed by the low magnitude of percent prediction error.

Key words: phenobarbitone, Orally disintegrating tablets, Eudragit®E100, Taste-masking, direct compression, Fractional factorial design

ACRONYMS

AEDs	Antiepileptic Drugs
ANOVA	Analysis of Variance
BNF	British National Formulary
CF	Compression Force
DEE	Drug Entrapment Efficiency
DT	Disintegration Time
EPHARM	Ethiopian Pharmaceutical Manufacturing Sh. Co.
FDA	United States Food and Drug Administration
FT-IR	Fourier-Transform Infrared
GABA	γ-aminobutyric acid
LAMIC	Low- and middle-income countries
MCC	Microcrystalline Cellulose
ODTs	Orally Disintegrating Tablets
PVA	Polyvinyl Alcohol
RS	Reference Standard
R2FI	Reduced two factors interaction
RSM	Response Surface Methodology
SD	Standard deviation
USP/NF	United State Pharmacopoeia / National Formulary
UV/Vis	Ultra Violet/Visible
WHO	World Health Organization

1. INTRODUCTION

1.1 Epilepsy

Epilepsy is a serious neurological condition characterized by recurrent seizures (Halliday *et al.*, 2012). Seizures may vary from the briefest lapses or muscle jerks to severe and prolonged convulsions. They may also vary in frequency, from less than once a year to several per day (WHO, 2003). Epilepsy is of worldwide public health importance because it is common, often accompanied by physical and cognitive disability and is widely stigmatized. Across the world and throughout history, epilepsy has been a culturally devalued condition. Such devaluing often leads to people with epilepsy being stigmatized and bearing a psychosocial burden (Boer *et al*., 2008). Patients with epilepsy often have seizures with impairment of consciousness and abnormal uncontrolled movements. In addition, they may have anti-epileptic drugs related side effects such as drowsiness, ataxia, and blurred vision. Therefore, these patients carry specific risk of injury during their daily activities (Boer *et al*., 2008; Bartolini *et al*., 2011; Asadi-Pooya *et al*., 2012; Camacho *et al.*, 2012). Studies show that, about 47% of patients with epilepsy report having at least one injury in 12 months. About 14.4% of patients reported having severe injuries.

1.1.1 Epidemiology: prevalence, incidence and mortality of epilepsy

The prevalence of epilepsy varies across studies, but generally ranges from 4 to 10 per 1000 population, being 2 to 25 times higher in the developing world (Swinkels *et al.*, 2005; Nizamiea *et al*., 2009). It is estimated that 50 million individuals worldwide have a diagnosis of epilepsy (Li *et al*., 2012). The World Health Organization (WHO) estimated that nearly 80% of the burden of epilepsy worldwide is borne by resource poor countries (Bartolini *et al.*, 2011). Moreover, it accounts for a worldwide illness burden similar to that of breast cancer in women and of lung cancer in men (Lv *et al*., 2011). In Ethiopia it affects an estimated 5.2 per 1000 of the population (Berhanu *et al*., 2009)

On average, the incidence of epilepsy is approximately 50 per 100, 000 population. Recent studies from Africa report the incidence of epilepsy in Tanzania to be 73 and in Ethiopia 64 (Hauser, 2003; Burneo *et al*., 2005).Its incidence occurs in age-dependent bimodal pattern, with an initial peak during the first year of life and another rise after the age of 60 (Gareri *et al*., 1999, Bagshaw *et al*., 2009).Worldwide, it is estimated that 10.5 million children under 15 years have active epilepsy, representing about 25% of the global epilepsy population. Of the 3.5 million people who develop epilepsy annually, 40% are younger than 15 years, and more than 80% live in developing countries. Population-based studies on childhood-onset epilepsy indicate annual incidence rates of 61–124 per 100, 000 in developing countries, and 41–50 per 100,000 in developed countries. Incidence falls progressively from around 150 per 100, 000 in the first year of life to 45–50 per 100, 000 after the age of 9 years (Guerrini, 2006). Elderly people (aged more than 60 years) have the highest prevalence and incidence of epilepsy and the prevalence keeps growing along with advancing age (Hauser, 2003). The annual incidence of epilepsy rises from 85 per 100, 000 people in those aged between 65 and 69 years to more than 135 per 100, 000 people for those aged older than 80 years (Brodie *et al*., 2009). Moreover, the risk of death for a person with epilepsy is increased compared with the risk for the general population

1.1.2 Treatment of epilepsy

Antiepileptic drugs (AEDs) are the mainstay of the treatment of epilepsy. The main treatment goals of patients with epilepsy are to control the seizures and to minimize drug side effects. Antiepileptic drugs provide satisfactory control of seizures for most patients with epilepsy. Recent studies in both developed and developing countries have shown that up to 70% of children and adults newly diagnosed with epilepsy can be successfully treated with antiepileptic drugs. The majority of patients can be successfully treated by monotherapy, although in some cases polytherapy is required in order to achieve satisfactory seizure control (Schmidt, 2009). However, about 30% of people with epilepsy do not attain full seizure control, even with the best available treatment regimen (Sweileh *et al*., 2011) . Resective surgery is a valuable option for the treatment of drug resistant epilepsy. Vagus nerve stimulation was approved for the treatment and prevention of refractory seizures in adults and adolescents by the European and US regulatory boards in 1994 and 1997, respectively (Romanelli et al., 2012). Moreover, ketogenic diet is also used in the treatment of epilepsy (Bailey et al., 2005)

Adherence to AED therapy is critical for effective disease management, yet adherence and persistence rates are low due to several barriers. Because epilepsy is so common, there are enormous public health consequences for any systematic treatment failure. Moreover, epilepsy is characterized by sporadic symptoms (i.e., seizures) rather than a continually perceptible phenomenon like pain, the patient is required to take medications which do not have immediately obvious benefit. The consequences of non-adherence may not appear immediately; thus, patients who miss doses without adverse results may conclude, erroneously, that rigorous medication adherence is not important. Furthermore, patients with epilepsy must take daily medication for years or for a lifetime (Faught, 2012)

Ongoing outpatient management of neonatal and pediatric seizures may be complicated by the lack of pediatric syrups or other formulations of AEDs appropriate for children. The shortage of pediatric AED formulations is due to higher cost and shorter shelf life of these agents compared to adult formations and may be further exacerbated by the scarcity of trained pharmacists to prepare and dispense pediatric formulations (Birbeck, 2010)

1.2 Phenobarbitone

Phenobarbitone, introduced in 1912 by Hauptmann, was the first synthetic drug to be used successfully to treat epilepsy (Zuppa *et al*., 2011). Since then, despite the development of successive generations of AEDs, phenobarbitone has retained a unique position in the therapeutic armamentarium and is still the most widely prescribed for treatment of epilepsy throughout the world. It is an effective major antiepileptic drug, used in the treatment of tonic-clonic seizures and simple and complex partial seizures except for absence (petit mal) seizures (Bruni, 1979). It has also sedative and hypnotic properties, which will help patients to relax before surgery or help to sleep (Jelveghari and Nokhodchi, 2008).

Phenobarbital would be ideal for use in resource poor countries, since it is cheap, readily available, fast acting and can be administered intramuscular at peripheral health facilities with few resources (Kokwaro *et al*., 2003). It has also advantages in terms of easy dosing (important not only for patients but also when non physician health care workers are prescribing and dispensing medications) and spectrum of activity (Birbeck, 2010). A study in rural India found that 65% of patients who received phenobarbitone were successfully treated, and that the same proportion responded to phenytoin. Adverse events were also similar in type and frequency in both groups. Studies in Ecuador and Kenya compared phenobarbitone to carbamazepine and found that there were no significant differences in either efficacy or safety. But, the cost of phenytoin, carbamazepine, and valproate are up to 5, 15, and 20 times as expensive, respectively (Scott *et al.*, 2001). Hence, it is recommended by the WHO as a first-line AED for partial and generalized tonic–clonic seizures in developing countries (Pal, 2006; Tonekaboni et al., 2006).

1.2.1 Physical and chemical properties

Phenobarbitone is a barbituric acid derivative, 5-phenyl-5-ethylbarbituric acid ($C_{12}H_{12}N_2O_3$; mol. wt. 232.24) (Fig. 1.1). It is a white, odorless crystalline powder which may exhibit polymorphism. Phenobarbitone has somewhat bitter taste and a melting point range of 174-178 ^{o}C. Its saturated solution has a pH of about 5. It is very slightly soluble in water (1 mg/ml); soluble in alcohol (100 mg/ml), in ether and in solution of fixed alkali hydroxides and carbonate; sparingly soluble in chloroform. It is also stable in air (USP30-NF25, 2007). Phenobarbitone is available as oral tablets (15, 30, 60 and 100 mg), capsules (16, 50 and 100 mg), elixir (15 mg/5 mL and 20 mg/5 mL) and intravenous injection (200 mg/ml) as phenobarbital sodium

Figure 1. 1 **: S**tructural formula of phenobarbitone.

1.2.2 Pharmacodynamic and pharmacokinetic

Phenobarbitone exerts it antiepileptic effect primarily by facilitating γ-aminobutyric acid (GABA) mediated inhibition via allosteric interaction with the neuronal postsynaptic $GABA_A$ receptors. It enhances the activation of $GABA_A$ receptors by increasing the mean channel open duration without affecting open frequency or conductance. The resultant increase in Cl^-flux hyperpolarizes the postsynaptic neuronal cell membrane, thus impeding the transmission of epileptic activity. At somewhat higher concentrations, phenobarbital can block sodium channels and may block excitatory glutamate response (Kwan and Brodie, 2004)

Oral absorption of phenobarbitone is complete but somewhat slow; peak concentrations in plasma occur several hours after a single dose. It is 40% to 60% bound to plasma proteins and bound to a similar extent in tissues, including brain. Up to 25% of a dose is eliminated by pH-dependent renal excretion of the unchanged drug; the remainder is inactivated by hepatic microsomal enzymes, principally CYP2C9, with minor metabolism by CYP2C19 and CYP2E1. Phenobarbital induces uridine diphosphate-glucuronosyl transferase (UGT) enzymes as well as the CYP2C and CYP3A subfamilies. Drugs metabolized by these enzymes can be more rapidly degraded when co-administered with phenobarbital; importantly, oral contraceptives are metabolized by CYP3A4 (Goodman and Gilman, 2006).

1.3 Orally disintegrating (dissolving) tablets (ODTs)

Orally disintegrating (dissolving) tablets (ODTs) are solid dosage forms that are placed in the mouth, rapidly disintegrate and/or dissolve when in contact with the saliva and then easily swallowed without the need for water (AlHusban *et al*., 2011). Over a decade, the demand for development of orally disintegrating tablets (ODTs) has enormously increased, as it has significant impact on the patient compliance. Orally disintegrating tablets offer an advantage for populations who have difficulty in swallowing. It has been reported that dysphagia (difficulty in swallowing) is common among all age groups of people, but it is more specific in pediatric and geriatric population (Aguilar-Díaz *et al.*, 2012).

Orally disintegrating tablets are also called orodispersible tablets, quick disintegrating tablets, mouth dissolving tablets, fast disintegrating tablets, fast dissolving tablets, rapid dissolving tablets, porous tablets, and rapimelts. However, of all the above terms, the United States Pharmacopoeia (USP) approved these dosage forms as orally disintegrating tablets or ODTs. The European Pharmacopoeia uses the term ‘‘Orodispersible tablet’’ for tablets that disperse readily within 3 min in mouth before swallowing. United States Food and Drug Administration (FDA) defined ODT as ‘‘A solid dosage form containing medicinal substance or active ingredient which disintegrates rapidly usually within a matter of seconds when placed upon the tongue’’ (Aguilar-Díaz *et al*., 2012, Pabari and Ramtoola, 2012)

Target groups for oral disintegrating tablets are wide ranging as people of all ages can experience difficulty in swallowing conventional tablets and capsules. This includes children and the elderly who either experience difficulty and cannot swallow or have not learnt to swallow the conventional solid dosage forms. In addition, institutionalized psychiatric patients as well as hospitalized or bedridden patients suffering from a variety of disorders such as stroke, Parkinson’s disease and other neurological disorders such as multiple sclerosis and cerebral palsy also find difficulty in swallowing and require oral disintegrating tablets because of their physical condition. The convenience and ease of using oral disintegrating tablets is also important to normal consumers, with some adults preferring these dosage forms as they are easy to handle and swallow, can be taken without water and have a rapid onset of action. For example, patients with a limited access to water would also find such ODTs extremely beneficial (Chandrasekhar *et al.*, 2009; Peera *et al*., 2013).

ODTs provide several advantages as they combine the properties of both liquid and conventional tablet formulations (Gryczke *et al.*, 2011), and also offering advantages over both traditional dosage forms. ODTs are unit solid dosage forms, they provide good stability, accurate dosing, easy manufacturing, small packaging size, and easy to handle by patients; no risk of obstruction of gastrointestinal tract by the dosage form, which is beneficial for traveling patients who do not have access to water, easy administration of inpatients (specially for mentally retarded and psychiatric patients); the rapid disintegration of the tablet results a quick dissolution of the drug and fast absorption that provide rapid onset of action (Aguilar-Díaz *et al*., 2012). Besides improving the acceptability and compliance of patients, ODTs have been investigated for their potential to increase the bioavailability through the enhancement of the dissolution rate (Rahman *et al*., 2010)

1.3.1 Manufacturing methods

There are several technologies that produce orally disintegrating tablets. The technologies are usually grouped according to the method used in making orally disintegrating tablets: freeze drying method, molding method, and compression method (Fu *et al*., 2005). ODT preparation methods also include sublimation, spray-drying, phase transition and mass extrusion.

1.3.1.1 Freeze drying

Freeze drying (lyophilization) is a process in which solvent is removed from a frozen drug solution or a suspension containing structure-forming excipients. The resulting tablets are usually very light and have highly porous structures that allow rapid dissolution or disintegration (Bhasin *et al*., 2011). One of the main advantages of this method is, when stored in a dried state, the freeze-dried dosage form has relatively few stability problems during its shelf life. The freeze-drying process may result in a glassy amorphous structure of excipients as well as the drug substance, leading to the enhanced dissolution rate. Freeze drying, however, is a relatively expensive manufacturing process, and the formulation has poor stability at higher temperature and humidity (Bircan and Çomoğlu, 2012).

1.3.1.2 Direct compression

Direct compression is one of the popular techniques for preparation of ODTs. The advantages of this method include easy implementation, use of conventional equipments along with commonly available excipients, limited number of processing steps, cost effectiveness and also accommodate higher dose in comparison to the other methods

(Agrawal *et al*., 2011). The basic principle involved in development of these dosage forms using this technique is addition of superdisintegrants in optimum concentrations so as to achieve rapid disintegration along with pleasant mouth feel. The disintegration and dissolution of directly compressed tablets depends on single or combined effect of disintegrant, water soluble excipients and effervescing agents. Disintegrant efficacy is strongly affected by tablet size and hardness (Nayak and Manna, 2011). Sugar-based excipients have been widely used as bulking agents because of their high aqueous solubility and sweetness, pleasant mouth-feel and good taste-masking capacity. Nearly all formulations for ODTs incorporate some sugar materials in their formulations (Udaykumar *et al*., 2012).

1.3.1.3 Molding

In this method, molded tablets are prepared by using water-soluble ingredients so that the tablets dissolve completely and rapidly. The powder blend is moistened with a hydro-alcoholic solvent and is molded into tablets under pressure lower than that used in conventional tablet compression (Badgujar and Mundada, 2011). The solvent is then removed by air drying. Molded tablets are very less compact than compressed tablets. These possess porous structure that enhances dissolution (Fu *et al.*, 2005).

1.3.1.4 Sublimation

This technique is based on the use of volatile ingredients (e.g. camphor, ammonium bicarbonate, naphthalene, urea, urethane etc.) to other tablet excipients and the mixture is then compressed into tablets. Entrapped volatile material is then removed via sublimation (Badgujar and Mundada, 2011). A high porosity is achieved due to the formation of many pores where volatile material particles previously existed in the compressed tablets prior to sublimation of the volatile ingredient. These compressed tablets which have high porosity (approximately 30%) rapidly dissolve within 15 sec in saliva (Prajapati and Ratnakar, 2009)

1.3.1.5 Spray drying

Spray drying provides a fast and economical way of removing solvents and producing highly porous fine powders that dissolve rapidly (Ashish *et al*., 2011). This technique is based on a particulate support matrix, which is prepared by spray drying an aqueous composition containing support matrix and other components to form a highly porous and fine powder. This is then mixed with active ingredients and compressed into tablets. The formulations are incorporated by hydrolyzed and non-hydrolyzed gelatins as supporting agents, mannitol as bulking agent, sodium starch glycolate or crosscarmellose sodium as disintegrating and an acidic material (e.g., citric acid) and/or alkali material (e.g., sodium bicarbonate) to enhance disintegration and dissolution (Nayak and Manna, 2011).

1.4 Taste- masking in ODTs

The taste-masking of unpleasant taste drugs is a major challenge especially for the development of orally disintegrating tablets in pharmaceutical industry to achieve patient acceptability (Maniruzzaman *et al*., 2012). Taste of a pharmaceutical product is an important parameter for governing compliance. Thus, taste-masking of oral pharmaceuticals has become an important tool to improve patient compliance and the quality of treatment, especially in pediatrics (Bhoyar *et al*., 2012, Guhmanna *et al*., 2012).

Several methods are available to mask undesirable taste of drugs (Gupta *et al*., 2012). The first is to mask the distasteful sensation by the addition of flavors, sweeteners and effervescent agents. The second method involves the use of complexing agents (cylcodextrins, ion exchange resins) through the formation of inclusion complexes or resinates. The third is to avoid the bitter drugs coming into direct contact with patients' taste buds by coating or granulation. The flavor is often over powered by the taste of the medicine and the use of effervescent agents is not always convenient. Moreover, the coatings and the granulation of the active ingredient may often rupture during compressing and chewing of the tablet, as well as contribute to a gritty feel. In recent years, microspheres and microencapsulations have been developed for taste-masking by creating a physical barrier to protect the bitter drugs from coming in contact with the patients' taste buds. It has also been reported that these microparticles remained intact without undergoing merging or rupturing during tabletting (Xu *et al*., 2008). Solvent evaporation is a relatively simple and convenient method for the preparation of taste-masked microspheres (Shah *et al*., 2012).

Taste-masking is achieved using the pH sensitive polymer Eudragit® E100. Eudragit® E100 is a cationic polymer based on dimethylaminoethyl methacrylate and other neutral methacrylic acid esters (butyl methacrylate, and methyl methacrylate) with a ratio of 2:1:1 (Dhoka *et al*., 2011). It is soluble in gastric fluids as well as in weakly acidic buffer solutions up to pH 5 by formation of salts but insoluble at salivary pH (Anand *et al*., 2007).

1.5 Optimization methods

Pharmaceutical formulators often face the challenge of finding the right combination of formulation variables that will produce a product with optimum properties. One of the difficulties in the quantitative approach for formulation design is due to difficulty in understanding the real relationship between causal factors and individual pharmaceutical responses. Optimization becomes more important for particular delivery systems (Ravi *et al*., 2010).

The traditional 'one variable at a time method' requires many experiments and there is no guarantee that an optimal formulation can be achieved. Moreover, the interaction between different factors, which can influence the target responses, may not be detected (Leesawat *et al*., 2004). But, statistical experimental design provides an economical means to obtain desirable information about an experimental method by just performing a few numbers of experimental runs; they also help to determine any type of interactions among study variables and also used to predict the chances of experimental errors (Ranjan *et al*., 2012, Mahmood *et al*., 2014). Therefore, the use of an experimental design is quite helpful in the optimization of pharmaceutical formulations.

Response surface methodology (RSM) is a collection of mathematical and statistical methods that is used to develop, improve, or optimize a product or process. It comprises statistical experimental designs, regression modeling techniques, and optimization methods. Most applications of RSM involve experimental situations where several independent (or control) variables potentially impact one (or more) response variable. The independent variables are controlled by the experimenter, in a designed experiment, while the response variable is an observed output of the experiment. RSM is the best method for parameters optimization with reduced number of experiments without affecting the accuracy of results (Leesawat *et al*., 2004, Monajjemzadeh *et al*., 2012).

The number of experiments in an experimental design grows rapidly with increasing number of variables. When dealing with many variables, a full factorial design is not a realistic option. A reduced design, called a fractional factorial design, is then a more appropriate option. For a fractional factorial design, experiments are chosen to give the maximum amount of variation with fewer experimental runs. The drawback is loss of information caused by confounding of main effects with interaction effects if the resolution is lower than four (Kumar and Karimulla, 2012).

1.6 The present study

It has been reported that dysphagia is common among all age groups of people. However, pediatric and geriatric patients are the most affected (Aguilar-Díaz *et al*., 2012). According to different studies, from 26 % to50% of patients find it difficult to swallow conventional tablet and capsule (Reig *et al.*, 2006).This leads to patient non-compliance and potentially prolonged duration of treatment.

Adherence to antiepileptic drug (AED) therapy is critical for effective disease management (Faught, 2012). However, AED non-adherence is highly prevalent; with estimates ranging from 20 to 80%. Non-compliance is a major reason for treatment failure and can have serious consequences. It contribute to increased direct and indirect healthcare costs related to epilepsy (Hovinga *et al*., 2008, Nakhutina *et al*., 2011 Sweileh *et al*., 2011). One of the factors affecting treatment compliance is the pharmaceutical form used. But, the pharmaceutical industry supplies oral solid dosage forms that are generally inadequate for pediatric, geriatric and dysphagia patients needs. The only approved oral alternative, phenobarbital oral elixir, described in the BNF contains a high amount of alcohol (38%) which can increase possible unpleasant effects (Jelveghari and Nokhodchi, 2008). For instance regular administration of this elixir may cause alcohol toxicity, especially in neonates. Thus, Phenobarbitone elixir, which containing a high amount of alcohol is not recommended for children (Djabri *et al*., 2012)

The development of an appropriate dosage form for older people, children, and bedridden patients has been widely desired because it can be difficult for these patients to swallow conventional tablets or capsules. Hence, formulation of orally disintegrating phenobarbitone tablets could help to improve patient compliance and therapeutic effect. Moreover, unpleasant effect caused by high concentration of alcohol contained in phenobarbitone oral elixir is avoided.

Hence, the aim of the current study is to formulate taste-masked Phenobarbitone orally disintegrating tablets, which is suitable for pediatric, geriatric and dysphagia patients

1.7 Objectives

1.7.1 General objective

To formulate and optimize taste-masked phenobarbitone orally disintegrating tablets (ODTs)

1.7.2 Specific objectives

- To determine the bitterness threshold concentration of phenebarbitone *in vivo*
- To prepare and characterize taste-masked phenobarbitone microspheres
- To conduct drug excipient interaction study
- To investigate the effect of various formulation and process variables on tablet characteristics
- To formulate, characterize and optimize taste-masked phenobarbitone ODTs using appropriate optimization technique and validate the optimized formulation
- To characterize the optimized taste-masked phenobarbitone ODTs.

2. EXPERIMENTAL

2.1 Materials

Phenobaribitone (Jiangsu, Lianyungang, China), Eudragit® E100 (Röhm GmbH, Darmstadt, Germany), were kindly donated by Addis Pharmaceutical Factory S.C., Ethiopia. Microcrystalline Cellulose (Avicel R 102, NB, Entrepreneurs. Nagpur, India). Sodium Starch Glycolate (China Associate Co. Ltd, Shenzhen, China), Colloidal Silicon Dioxide (aerosil®)(Wacker Silicon, Germany) Magnesium Stearate (China Associate Co. Ltd, Shenzhen China), Mannitol (China Associate Co. Ltd, Shenzhen, China). Ethanol (Loba Chemie Pvt.LTD., Mumbai, India), Dichloromethane (BDH Ltd., Poole, England), Polyvinyl Alcohol (PVA)(BDH Ltd., Poole, England), Hydrochloric Acid and Sodium Hydroxide (BDH Ltd., Poole, England), Potassium chloride (BDH Ltd., Poole, England), Boric acid (Loba Chemie Pvt.LTD., Mumbai, India). Potassium phosphate monobasic (BDH Ltd., Poole, England) were all used as received. Phenobarbitone 30 mg oral tablet (EPHARM, Addis Ababa, Ethiopian) was purchased from the local market.

2.2. Methods

2.2.1 Construction of calibration curve

2.2.1.1 Calibration curve of phenobarbitone in pH 9.6 borax buffer

Twenty milligram of USP phenobarbitone RS was accurately weighed using an analytical balance (AAA analytical balance, ae Adam Equipment, Maidstone UK), transferred to a 100 ml volumetric flask containing 10 ml of ethanol and was diluted with borax buffer pH9.6 to volume. Standard solutions of final concentration of 4, 6, 10, 16, 18 and 20 µg/ml were prepared in 50 ml volumetric flask. Then the absorbance of the resulting solution were measured spectrophotometrically (CM2203, SOLAR, Minsk, Belarus) at λ max of 240 nm (James and Elmer, 1968). Alkaline borax buffer (pH 9.6) was used as a blank. Then, the absorbance versus concentration of solutions were plotted to obtain the calibration curve.

2.2.1.2 Calibration curve of phenobarbitone in 0.1 N HCl

Stock solution of phenobarbitone reference standard was prepared by transferring 20 mg of phenobarbitone reference standard to a 100 ml volumetric flask containing 10 ml of ethanol and diluting with a mixture of 0.1N hydrochloric acid and 0.1N sodium hydroxide (1:2) to volume. From this stock solution, seven different standard solutions of final concentration of6, 10, 14, 18, 24, 26, and 28 µg/ml were prepared. The UV absorbance readings of these solutions were measured at 240 nm using UV/Visible spectrophotometer (CM2203, SOLAR, Minsk, Belarus) (O'Neil, 2006). A mixture of 0.1N hydrochloric acid and 0.1N sodium hydroxide (1:2) was used as a blank. Then, the absorbance versus concentration of solutions were plotted to obtain the calibration curve.

2.2.1.3 Calibration curve of phenobarbitone in pH 6.8 Phosphate buffer

Stock solution of phenobarbitone reference standard was prepared by transferring 20 mg of phenobarbitone reference standard to a 100 ml volumetric flask containing 10 ml of ethanol and diluting with a mixture of Phosphate buffer (pH = 6.8) and with borax buffer (pH9.6)(1:2)to volume. From this stock solution, six different standard solution of final concentration of 9, 15, 21, 30, 36 and 40 µg/ml were prepared. The UV absorbance readings of these solutions were measured at 240 nm using UV/Visible spectrophotometer (CM2203, SOLAR, Minsk, Belarus) **(**O'Neil, 2006). A mixture of Phosphate buffer (pH 6.8) and borax buffer (pH 9.6) (1:2) was used as a blank. Then, the absorbance versus concentration of solutions were plotted to obtain the calibration curve.

2.2.2 Determination of bitterness threshold concentration of phenobarbitone

The bitter taste threshold value of Phenobarbital was determined using the method described by (Kulkarni *et al.*, 2012) based on taste recognition by six volunteers from whom informed consent was obtained. A series of phenobarbitone standard solutions of different concentrations (25, 50, 75, 100, 125, 150, 175, and 200 μg/ml) were prepared in phosphate buffer pH 6.8. Starting with the lowest concentration, the volunteers were instructed to place 1ml of the standard solution on the center of the tongue. The solution was retained in the mouth for 30 sec and then the mouth was thoroughly rinsed with distilled water. The next higher concentration was then tasted after 10 min.

2.2.3 Preparation of taste-masked phenobarbitone microspheres

Various microspheres were formulated using emulsion solvent evaporation method as described by Dhoka *et al.*, (2011). At first, 10 gram different ratio of the Eudragit® E100 and drug (1:1, 2:1, 3:1, 4:1) were co-dissolved in 40 ml mixture of ethanol and dichloromethane (1:1, v/v). Then, the drug solution was slowly injected using a 10 ml hypodermic syringe into a 400 ml 1% aqueous solution of polyvinyl alcohol (PVA) and was stirred on a thermostatic magnetic stirrer (VELP Scientifica, Usmate Velate, Italy) at various stirring rates (500, 650 and 800 rpm) at 25 ^{o}C for 1 h to allow the volatile solvent to evaporate completely**.** The microspheres formed were finally filtered, washed three times using distilled water, filtered, and then dried overnight.

2.2.4 Characterization of prepared microspheres

2.2.4.1 Particle size and size distribution

The particle size and size distribution of the microspheres were determined using sieving method USP30/NF25 (2007). Weighed microspheres of each formulation were put in a set of sieves fixed on the universal drive unit (ERWEKA, AR 402, Germany). Microspheres that were retained on each sieve were collected and weighed, and the average particle size was analyzed based on Eq. 2.1.

$$Average\ particle\ size = \frac{\sum\left[\%\,\mathrm{Re}tained \times d_i\right]}{100} \qquad \text{Eq.2.1}$$

Where d_i is the arithmetic mean of the upper and lower openings of the sieve on which the portion of microspheres was retained (Ansel and Allen, 2014).

2.2.4.2 Bulk density

For each formulation, 20 g of microsphere was carefully introduced into a 250 ml graduated cylinder and the volume of the microspheres was noted. The bulk density of each formulation was then obtained by dividing the weight of samples by their respective volume using Eq. 2.2.

$$D_b = \frac{M}{V_b} \qquad \text{Eq.2.2}$$

Where, D_b is bulk density (g/cm^3), M is weight of sample (g), and V_b is volume of microspheres (cm^3).

2.2.4.3 Tapped density

A sample of 20 g of microspheres was carefully introduced into a 250 ml graduated cylinder and was tapped 250 times using a tap densitometer (ERWEKA, SVM 20, Germany). The tapped volume was then noted and the tapped density of each formulation was calculated using Eq. 2.3.

$$D_t = \frac{M}{V_t} \qquad \text{Eq.2.3}$$

Where D_t is tapped density (g/cm^3), M is weight of sample (g), and V_t is final tapped volume of powder (cm^3).

2.2.4.4 Carr's index (Compressibility index)

The Carr's index of each formulation was calculated from bulk and tapped densities using Eq. 2.4.

$$Carr's\ index\ (\%) = \frac{(D_t - D_b)}{D_t} \times 100 \qquad \text{Eq. 2.4}$$

Where, D_b is bulk density (g/cm^3), and D_t is tapped density (g/cm^3).

2.2.4.5 Angle of repose

Angle of repose was determined by using a fixed funnel method. Accordingly, 20 g of the sample was allowed to flow from 10 cm height through a glass funnel orifice with an inner diameter of 15 mm. Then the radius (R) and the height (H) of the pile were determined and the angle of repose (θ, degree) was calculated using Eq. 2.5.

$$Angle\ of\ repose\ (\theta) = \tan^{-1}\left(\frac{H}{R}\right) \qquad \text{Eq. 2.5}$$

2.2.4.6 Percentage yield of microspheres

The production yield of microspheres of each batch was calculated as described by (Singh and Chaudhary, 2011) using the weight of the final product after drying with respect to the initial total weight of the drug and polymers used for preparation of microspheres, and the percentage production yield was calculated using Eq. 2.6.

$$Yield\ (\%) = \frac{\text{Pr}actical\ mass(microspheres)}{Theoretical\ mass(polymer + drug)} \times 100 \qquad \text{Eq.2.6}$$

2.2.4.7 Drug entrapment efficiency

Drug entrapment efficiency (DEE) was determined using the method described by Dhoka *et al*. (2011). Accordingly, a sample of 30 mg drug loaded microspheres of each formulation was taken for evaluation. The weighed microspheres were dissolved in 10ml ethanol and the amount of drug within the sample was assayed using a UV-Visible spectroflorimeter (CM2203, SOLAR, Minsk, Belarus) at 240 nm after appropriate dilution with pH 9.6 borax buffer. The amount of drug entrapped in the microspheres was calculated by Eq. 2.7.

$$DEE\ (\%) = \frac{Actual\ drug\ content}{Theoretical\ drug\ content} \times 100 \qquad \text{Eq.2.7}$$

2.2.5 *In vitro* taste-masking evaluation of microspheres

Taste-masking evaluation of microspheres was determined *in vitro* using the method described by (Malik *et al*., 2011). Microspheres (equivalent to 30 mg of phenobarbitone) were placed in a volumetric flask with 25 ml of phosphate buffer pH 6.8 and stirred for 5 min. The mixture was filtered, and then the concentration of the drug in the filtrate was analyzed and compared with the threshold value determined *in vivo*.

2.2.6 Drug-excipient interaction study

Drug-excipient interaction study was carried out using an FT-IRspectroscopy. FT-IR spectra of phenobarbitone, Eudragit® E100, and 1:1 physical mixture of phenobarbitone Eudragit® E 100 were recorded at room temperature using an FT-IR spectrophotometer (FTIR-8400S, Shimadzu, Japan) in transmittance mode. During the study 5-10 mg of the sample was ground and mixed with an oily mulling agent (Nujol) in a mortar and pestle. The mixture was then placed onto the face of a potassium bromide (KBr) plate and a second window was placed on top of the first salt plate to form a thin film of the mull by compression between two plates. The compressed plate was then placed in the infrared spectrometer and the spectra were recorded. Each IR spectrum was collected with 20 scans and spectral resolution of 4 cm^{-1}. Scanning was performed between wave numbers 4000 - 400 cm^{-1}. Background spectrum was collected before running each sample. IR Solution Software was used for data analysis.

2.2.7 Physicochemical characterization of a powder blend for tablet compression

The physicochemical properties of various powder blends, such as bulk and tapped density, compressibility index, angle of repose and Hausner ratio were determined using the methods described under section 2.2.4.2 - 2.2.4.5

2.2.7.1 Compression of plain and taste masked orally disintegrating phenobarbitone tablets

A. Compression of orally disintegrating phenobarbitone tablets

Plain orally disintegrating phenobarbitone tablets were prepared by direct compression method as per the formula (Table 2. 1). During the preparation all the ingredients were passed through a 224 µm sieve separately and all the formulations, except the lubricant and glidant, were mixed in a TURBULA® Mixer (Willy A. Bachofen AG, Turbula®2TF, Basel, Switzerland) at a speed of 49 rpm for 10 min. Then lubricant and glidant were added and mixed for further 5 min. Finally, the blend obtained was directly compressed using a single punch tablet press (KORSCH, Berlin, Germany), a 10 mm round, flat-faced tooling's.

Taste-masked phenobarbitone ODTs were also directly compressed following the same method as that of plain ODTs (at 10 and 17 KN compression force). However, in taste-masked ODTs the phenobarbitone loaded microspheres (Table 2.2) were used in place of the pure drug without sieving.

Table 2. 1: Compositions of different plain fast disintegrating tablets of phenobarbitone.

Formulation	MCC:mannitol	Compositions of Ingredients (% W/W)					
		phenobarbitone	MCC	Mannitol	SSG	Aerosil	Mg stearate
F1	2:1	12	55.00	27.50	4	0.5	1.0
F2	2:1	12	52.30	26.20	8	0.5	1.0
F3	2:1	12	49.70	24.80	12	0.5	1.0
F4	1:1	12	41.25	41.25	4	0.5	1.0
F5	1:1	12	39.25	39.25	8	0.5	1.0
F6	1:1	12	37.25	37.25	12	0.5	1.0
F7	1:2	12	27.50	55.00	4	0.5	1.0
F8	1:2	12	26.20	52.30	8	0.5	1.0
F9	1:2	12	24.80	49.70	12	0.5	1.0
F10	1:3	12	20.63	61.87	4	0.5	1.0
F11	1:3	12	19.63	58.87	8	0.5	1.0

Table 2. 2: Composition of different taste masked ODTs of phenobarbitone.

Formulation	MCC:mannitol ratio	polymer:drug ratio	Composition of Ingredients (% W/W)					
			Phenobarbitone Loaded microsphere	MCC	Mannitol	SSG	Aerosil	Mg Stearate

OF1	1:1	3:1	25	34.75	34.75	4	0.5	1.0
OF2	1:1	1:1	25	34.75	34.75	4	0.5	1.0
OF3	1:1	3:1	25	32.75	32.75	8	0.5	1.0
OF4	2:1	3:1	25	46.33	23.17	4	0.5	1.0
OF5	2:1	3:1	25	43.67	21.83	8	0.5	1.0
OF6	2:1	1:1	25	43.67	21.83	8	0.5	1.0
OF7	1:1	1:1	25	32.75	32.75	8	0.5	1.0
OF8	2:1	1:1	25	46.33	23.17	4	0.5	1.0

2.2.8 Tablet characterization

2.2.8.1 Crushing strength

Ten tablets were randomly taken and the crushing strength of each tablet was determined using a hardness tester (CALEVA. Model; THT2, Frankfurt Germany) 24 h after compression. The mean value and standard deviation of the ten tablets were recorded.

2.2.8.2 Friability

Ten tablets were randomly selected from each lot weighed and placed in a friability tester (ERWEKA, Model; TAR 20, Germany). The tablets were made to rotate in the chamber at a speed of 25 rpm for 4 min. Then, the tablets were de-dusted using a 2 mm sieve and reweighed. The percentage weight loss was calculated as percentage friability using Eq. 2.8.

$$\text{Percentage Friability} = \frac{(W1 - W2)}{W1} \times 100 \qquad \text{Eq. 2.8}$$

Where, W1 = Initial weight of the 10 tablets, W2 = Final weight of the 10 tablets after testing.

2.2.8.3 *In vitro* disintegration test

In vitro disintegration time was carried out according to USP XXVII. Six tablets from each formulation were randomly selected and placed in a disintegration apparatus (GB Caleva Ltd., Model; DIST2, England) filled with 800 ml of distilled water kept at 37 ± 2 ^{o}C. The time required for complete disintegration of the tablets with no palpable mass remaining in the apparatus was recorded as the disintegration time.

2.2.8.4 Wetting time

Wetting time of the ODTs was determined according to methods described by Nayak and Manna, 2011, Nasser *et al.*, (2012) . A piece of tissue paper (12 cm x 10.75 cm) folded twice was placed in a small petridish (ID = 9 cm) containing 10 ml of distilled water and amaranth (0.2% w/v). Then the tablet was carefully placed on the upper surface of the tissue paper and the time needed for the tablet to become completely red was recorded as wetting time. Six tablets from each formulation were randomly selected, and the mean value and standard deviation were calculated.

2.2.8.5 *In vitro* dissolution studies

In vitro dissolution studies were carried out using USP apparatus type II. An automated dissolution tester (ERWEKA DT 600HH, GERMANY) at 50 rpm, and a dissolution medium of 900 ml 0.1 N HCl maintained at 37 ± 0.5 °C was used. 10 ml aliquots of dissolution media were withdrawn at 5, 10, 15, 20, 30, 45 and 60 min intervals. Each sample was filtered through a Whatmann filter paper No.1 and suitably diluted with 0.1N sodium hydroxide and filtered again. The volume withdrawn at each time point was replaced with equal quantity of fresh dissolution medium. The content of phenobarbitone was spectrophotometrically analyzed at 240 nm. Cumulative percent of phenobarbitone released was calculated and plotted against time. For each batch 6 tablets were tested.

2.2.8.6 Drug content and content uniformity test

The content of phenobarbitone was determined according to the method described by Malviya, (2012) with slight modification. Ten tablets were powdered and 30 mg equivalent weight of phenobarbitone in tablet powder was accurately weighed and transferred into a 100 ml volumetric flask. Initially, 10 ml methanol was added and shaken for 10 min. Then, the volume was made up to 100 ml with borax buffer pH 9.6. Subsequently, the solution in volumetric flask was filtered and 1 ml of the filtrate was diluted and analyzed at 240 nm using UV-visible spectrofluorimere (CM2203, SOLAR, Minsk, Belarus).The drug content was determined using the standard calibration curve. For content uniformity 10 tablets were randomly selected from each batch and were analyzed individually. The amount of phenobarbitone was analyzed spctrophotometrically.

2.2.9 Experimental design

Preliminary experiments indicated that variables such as compression force (A), percentage of disintegrant (B), MCC to mannitol ratio (C) and drug to polymer ratio (D) were found to be the critical factors which affect the various responses. The response variables investigated were the disintegration time (DT), wetting time, tablet hardness, drug release within 5 min and 15 min. Fractional factorial design was adopted to statistically optimize the responses. In this design the above 4 factors were studied at 2 levels performing a total of 8 experiments (i.e. $2^{4-1} = 8$). The results of optimization experiments were analyzed using Design-Expert 8.0.4 software where the optimum area at which the desired responses were achieved, and to the response surface plots and contour plots for the fitted polynomial equations of the responses were obtained. The selected formulation and process variables with their limits, units and notations are given in Table 2.3.

Table 2. 3: Independent variables and their levels.

Variables	Levels	
	-1	+1
Compression force, A (KN)	10	17
Sodium starch glycolate, B (%)	4	8
MCC:Mannitol, C (w/w)	1	2
Polymer: drug, D (w/w)	1	3

2.3 Statistical analysis

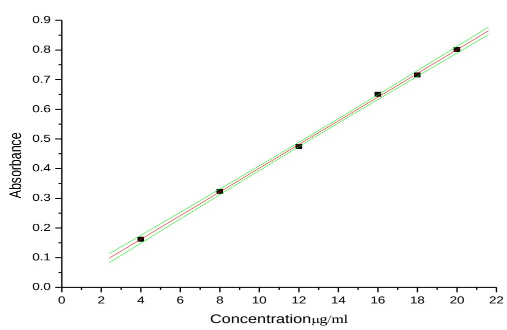
Unless and otherwise stated, each test was conducted in triplicate and the results are reported as mean and standard deviation. The results were treated statistically using Origin 7 software (OriginLab Corporation, MA, and USA). One way ANOVA was applied for comparison of results. A statistically significance difference was considered when $P < 0.05$.

3. RESULTS AND DISCUSSION

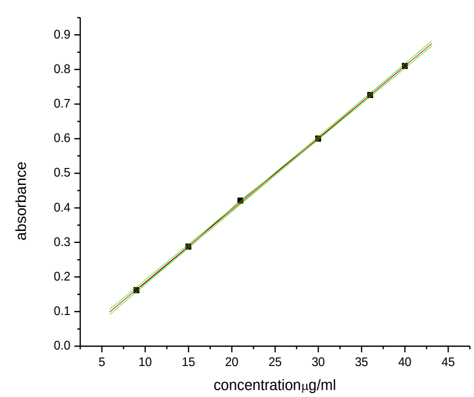
3.1 Calibration curves of phenobarbitone

The UV absorbance calibration curve of pure phenobarbitone at 240 nm in Borax buffer pH 9.6, 0.1N HCl, Phosphate buffer pH 6.8 are shown in Figure 3.1A, B and C , respectively

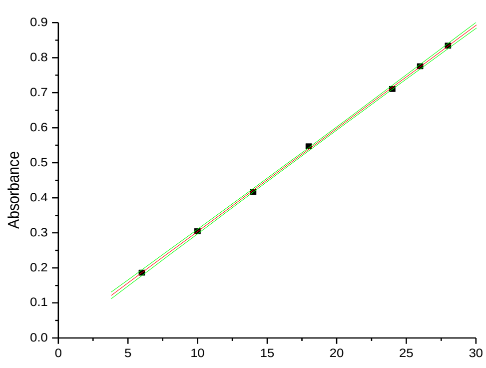
The Beer Lambert calibration curve (Figure 3.1 A, B and C) yielded a linear regression equation of A= 0.03992 C + 0.00232, A= 0.0294 C + 0.0098 and A= 0.020 C + 0.023 (where A is the absorbance and C is the concentration in *μg/ml* with 95% confidence interval) and coefficient of determination (R^2) of 0.9998**,** 0.9997 and 0.9998, p<0.0001**)** respectively.



A



C



B

Figure 3.1: UV absorption calibration curve of phenobarbitone reference standard in pH 9.6 Borax buffer (A), 0.1N HCl (B) and Phosphate buffer pH 6.8 (C) at 240 nm with 95% confidence interval

3.2 Bitterness threshold concentration of phenobarbitone

The bitterness threshold concentration of phenobarbitone was determined *in vivo* on six healthy human volunteers with series of phenobarbitone standard solutions of different concentrations (25, 50, 75, 100, 125, 150, 175, and 200 µg/ml). Five out of six volunteers felt the bitterness, after 30 sec at the concentration of 150 µg /ml and only one of the volunteers not felt the bitterness at 150 µg /ml. Therefore, it was concluded that the threshold concentration of phenobarbitone that triggered the sensation for bitterness, was taken to be 150 µg/ml.

3.3 Characteristics of taste-masked phenobarbitone microspheres

Taste is an important parameter in administering drugs orally and is a critical factor to be considered while formulating ODTs and other formulations which come in contact with the taste buds (Gupta *et al.*, 2010). Although there are different methods of taste masking, the entrapment of bitter drug substances in polymer based microspheres or microcapsule has become an increasingly attractive strategy for taste-masking by creating a physical barrier around the bitter drug to keep them from coming in direct contact with the patients' taste buds). Besides, these microparticles must remain intact without undergoing significant merging or rupturing during tabletting (Gupta et al., 2010; Yan et al., 2010).

Hence, in the current study microspheres with different polymer to drug ratios at different stirring rates were prepared using emulsion solvent evaporation technique and characterized. During the study, ethanol and dichloromethane (1:1) was used as the organic phase and 1% PVA solution as an aqueous continuous phase. The non-polar solvent dichloromethane was included to decrease the polarity of the polymer solution.

3.3.1. Particle size

The effects of polymer to drug ratio and stirring rate on microsphere particle size are presented in Table 3.1. As can be seen in the table, there is a significant increase in mean particle size ($p < 0.0001$) as the polymer to drug ratio increases. This finding is in agreement with the reports in the literature (El-Helw *et al.*, 2008).This might be due to the fact that as polymer concentration increases, viscosity also increases in a fixed volume of solvent and hence, leading to increase in microsphere size (Srivastava *et al*., 2005, Behera *et al*., 2008, Singh and Chaudhary, 2011). On the contrary, the mean diameter of the microsphere decreased significantly ($p < 0.05$) as the stirring speed increased. This could be attributed to the higher energy produced from the increased rate of stirring that further decreases the droplet sizes, thus producing smaller microspheres (Bari *et al*., 2010)

3.3.2 Drug entrapment efficiency and percentage yield

The effects of polymer to drug ratio and stirring rate on drug entrapment efficiency (DEE) and percentage yield were also investigated, (Table 3.1). As can be seen in the table, DEE increased significantly ($p < 0.05$) with increased polymer concentration. This has been rationalized by the fact that increasing in polymer concentration in the dispersed phase restricts the migration of the drug to the continuous phase, which consequently increases the entrapment efficiency (Freitas *et al*., 2005).

In contrast, entrapment efficiency (%) of the drug decreased with increasing stirring rate ($p < 0.05$). This is most likely attributed to the formation of small size microspheres with increased surface area which enhances drug diffusion. Besides the higher stirring rate on its own enhances the diffusion of drug from the microspheres to the aqueous solvent (Patel *et al*., 2012).

Observation of the effect of polymer to drug ratio on the percentage yield of the resulting microspheres showed that the percentage yield of the microspheres decreased with a decrease in the polymer to drug ratio. This is in agreement with those results reported by

Bansode *et al*., 2012; Shirolkar *et al.*, 2010 and can be associated to the loss of smallest and lightest particles during the filtration and washing processes (Abdallah *et al*., 2012). However, one-way ANOVA showed that the effect of stirring rate on the percentage yield was not statistically significant ($p > 0.05$).

Table : 3. 1: Characteristics of microspheres prepared at different stirring rates and polymer to drug ratios

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	P/D ratio	Stirring rate (rpm)	Yield % ± SD	Encapsulation Efficacy % ± SD	Drug release (µg/ml) after 5 min ± SD	Average paricle size ± SD (µm)	Bulk density g/ml ± SD	Tapped density g/ml ± SD	Angle of repose Degree ± SD	Hausner ratio± SD	Carr's index± SD
Fm1	1:1	500	57.40 ± 1.92	92.53±1.15	40.11 ± 1.72	423.58 ± 2.64	0.33 ± 0.02	0.37 ±0.02	30.20 ± 1.60	1.11 ± 0.01	9.91±0.49
Fm2	2:1	500	63.18 ± 2.26	94.38±1.72	35.22 ± 2.33	434.82 ± 4.11	0.31 ±0.03	0.35 ± 0.03	31.10 ± 1.11	1.12 ± 0.02	10.83±1.44
Fm3	3:1	500	65.78 ± 1.36	96.40±1.01	29.18 ± 1.94	445.18 ± 2.55	0.32 ±0.01	0.36 ± 0.01	30.22 ± 1.21	1.12 ± 0.02	10.76±1.88
Fm4	4:1	500	64.39 ± 1.21	97.00±1.26	21.95 ± 2.49	456.72 ± 3.53	0.30 ±0.03	0.33 ± 0.03	30.12 ± 1.21	1.10 ± 0.01	9.14±0.83
Fm5	1:1	650	54.35 ± 2.91	89.66 ± 2.06	58.07 ± 2.44	399.31 ± 4.82	0.32 ±0.03	0.35 ± 0.03	31.10 ± 1.11	1.12 ± 0.02	10.53±1.77
Fm6	2:1	650	62.11 ± 1.54	92.00 ± 2.01	49.25 ± 1.87	410.37 ± 4.06	0.31 ±0.03	0.35 ± 0.04	31.79 ± 1.53	1.13 ± 0.02	11.20±1.25
Fm7	3:1	650	63.89 ± 3.38	95.83 ± 1.98	37.93 ± 1.70	422.23 ± 5.03	0.31 ±0.01	0.35 ± 0.01	32.46 ± 1.60	1.13 ± 0.02	11.23±1.83
Fm8	4:1	650	64.09 ± 2.12	96.42 ±1.27	25.93 ± 2.16	435.01 ± 4.05	0.31 ±0.03	0.35 ± 0.04	32.15 ± 1.61	1.11 ± 0.01	10.07±1.01
Fm9	1:1	800	52.06 ± 2.30	88.50 ± 2.14	68.61 ± 2.42	386.01 ± 3.88	0.32 ±0.03	0.36 ± 0.02	33.23 ± 1.07	1.13 ± 0.03	11.80±2.22
Fm10	2;1	800	61.29 ± 1.34	91.35 ± 1.43	52.73 ± 2.74	398.28 ± 3.63	0.31 ±0.03	0.35 ± 0.03	33.15 ± 1.39	1.15 ± 0.03	13.25±1.97
Fm11	3:1	800	62.08 ± 1.26	94.31 ± 1.41	39.47 ± 3.39	410.81 ± 2.65	0.31 ±0.01	0.35 ± 0.02	32.03 ± 2.02	1.14 ± 0.01	12.51±0.52
Fm12	4:1	800	64.39 ± 1.21	95.23±1.31	29.69 ± 2.88	421.44 ± 3.19	0.31 ±0.02	0.35 ± 0.01	33.07 ± 1.70	1.14 ± 0.03	12.21±2.43

Fm: Formulation of microsphere, P/D: Polymer to drug ratio

3.3.3 Flow properties

The micromeritic properties of the prepared taste-masked phenobarbitone microspheres are shown in Table 3.1. As can be seen in the table, bulk density was found in the range of 0.30 ± 0.03 - 0.33 ± 0.02 g/cm^3 and the tapped density between 0.33 ± 0.03 - 0.37 ± 0.02 g/cm^3. Besides, all taste-masked microspheres had Hausner's ratio less than 1.25 indicating that they have good flow property (Basalious *et al.*, 2012). The compressibility index was found in the range of 9.14 ± 0.83 - 13.25 ± 1.97, which also indicates a good flowbility of the taste-masked microspheres. This is further substantiated by the values of angle of repose which was in the range 30.12 ± 1.21- 33.23 ± 1.07 ^{o}, indicating good flow characteristics of the microspheres. Besides, one-way ANOVA showed that both variables (polymer to drug ratio and stirring rate) had no significant effect on bulk and tapped densities, Hausner's ratio, Carr's index and angle of repose ($p > 0.05$).

3.3.4 *In vitro* taste-masking evaluation

The effects of polymer to drug ratio and stirring rate on the amount of drug released after 5 min are presented in Table 3.1. The results show that, as the polymer: drug ratio increased the release rate decreased significantly ($p < 0.05$). On the contrary, as the stirring rate increased, keeping other factors constant, microspheres exhibited increased drug release.

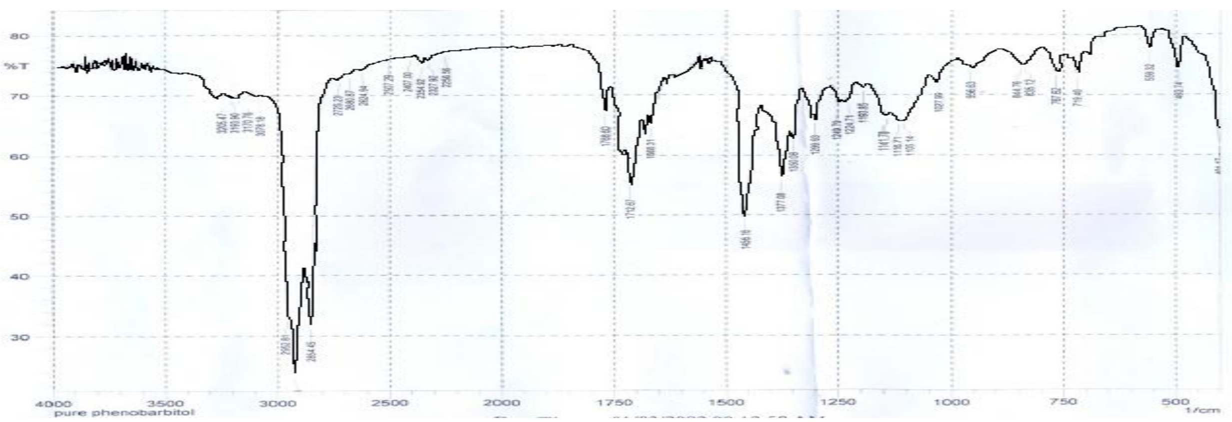
Moreover, the taste-masking efficiency of the phenobarbitone microspheres was determined by spectrophotometric analysis based on the amount of drug released after 5 min from the taste-masked microspheres in a pH 6.8 phosphate buffer, Table 3.1. As can be seen in the table, Fm 9 released the maximum amount of drug which was 68.61 ± 2.42 μg/ml. This value is less than half of the threshold bitterness concentration of 150 μg /ml of the drug. This might be attributed to the poor solubility of eudragit E100 in media with pH greater than 5 (Shishu *et al.*, 2009).

3.4 Compatibility studies

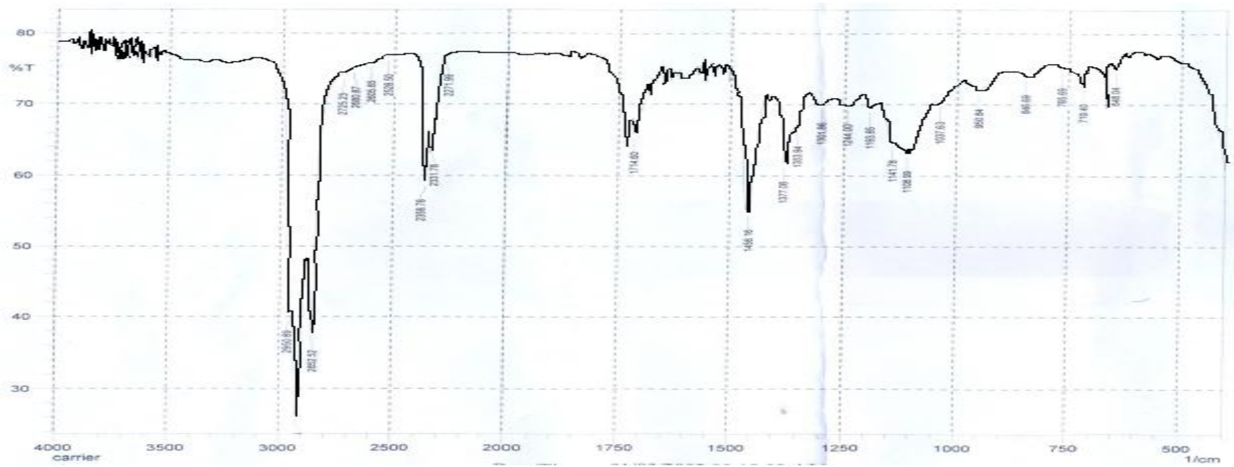
3.4.1 Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectra of phenobarbitone, Eudragit E® 100, and 1:1 physical mixture of phenobarbitone and Eudragit® E 100, respectively, are depicted in Figs 3.2 A to C.

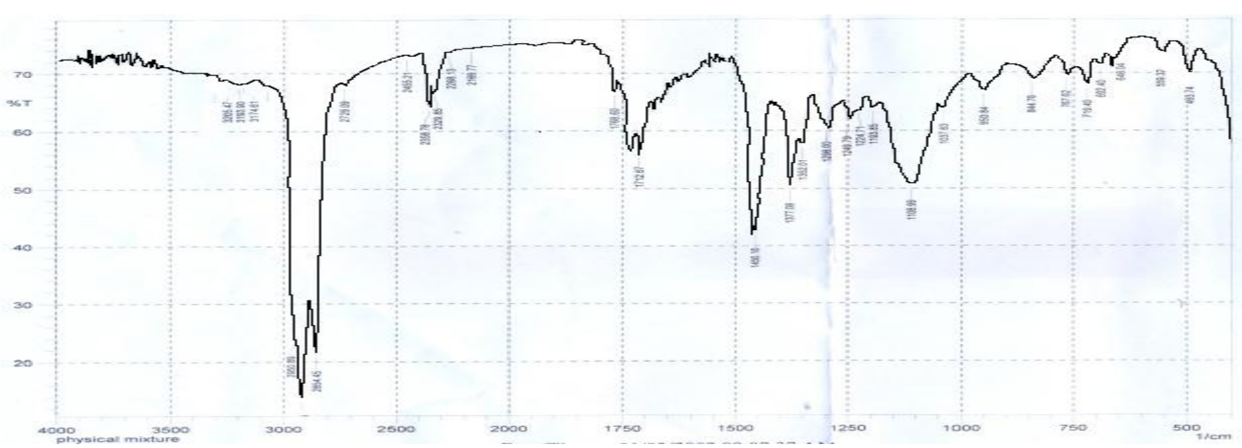
The FT-IR spectrum of phenobarbitone showed characteristic peaks at 3205.11 cm^{-1} and 3193.90 cm^{-1} for N-H stretching, 2950.89 cm^{-1}, 2923 $cm^{-1}$and 2854.45 cm^{-1} for C-H stretching of aromatics and alkane, respectively, 1712.67 cm^{-1} for C=O assymmetric stretching, 1456.16 $cm^{-1}$for C-C stretching of aromatic ring, 1350.08cm^{-1} and 1377.08 cm^{-1} for C-N asymmetric stretching (Gunasekaran *et al*., 2006). The FT-IR spectrum of the physical mixture of the drug and the polymer showed the characteristic peaks of phenobarbitone without any shift or reduction in intensity of peaks of phenobarbitone at, 3205.75 cm^{-1}, 3193.90 cm^{-1} for N-H stretching, 2950.89 cm^{-1} and 2854.45cm^{-1} for C-H starching of aromatic and alkane, 1712.67 cm^{-1} for C=O stretching, 1456.16cm^{-1} for C-C stretching (in aromatic ring) and 1377.08cm^{-1} for C-N asymmetric stretching thus suggesting that there is no interaction of drug with the components of the formulation occurred. The FT-IR spectrum of eudragit® E 100 showed characteristic peaks at 2,725.23 and 2,852.52 cm^{-1} for the dimethylamino groups, C-H vibrations can be discerned at 2,950.89 cm^{-1}, as well as the C=O ester vibration at 1,714.60 cm^{-1}. In addition, the FT-IR spectrum of the physical mixture showed no significant shift or reduction in intensity of the characteristic peaks of eudragit E 100 at 2,729.23 and 2,854.52 cm^{-1} for the dimethylamino groups, 2,950.89 cm^{-1}, for C-H vibrations and 1,712.60 cm^{-1} for the C=O ester vibration. Besides, there is no additional peak observed on physical mixture. Hence, the FT-IR spectroscopic study suggests that the drug is compatible with the polymer.



(A)



(B)



(C)

Figure 3. 2: FT-IR spectra of phenobarbitone (A) Eudragit® E100 (B) and 1:1physical mixture of phenobarbitone and Eudragit® E100(C).

3.5 Tablet formulation and characterization

Disintegration and solubilization of directly compressed tablets depend on single or combined action of disintegrants and water-soluble excipients. Consequently, SSG was used as superdisintegrant. Most commercial ODTs have been developed using mannitol as the bulk excipient of choice. It is overwhelmingly preferred over lactose because of its extremely low hygroscopicity, excellent chemical and physical compatibility, good compressibility and better sweetness (Patil *et al*., 2011). Thus, for this study it was used as a bulking agent. MCC is widely used in pharmaceutical formulations, primarily as a binder in tablets. It also has some lubricant and disintegrant properties that make it useful in tableting (Rowe *et al*., 2006). It also performs as a binder because it undergoes significant plastic deformation during compression bringing the extremely large surface area into closed contact and facilitating hydrogen bond formation between the plastically deformed, adjacent cellulose particles. In addition, the existence of moisture within the porous structure of MCC acts as an internal lubricant. This facilitates slippage and flow within the individual microcrystals during plastic deformation, which enforces the formation of hydrogen bond bridges and gives MCC a very good hardness (Yousuf *et al*., 2005). Therefore, it was used as a binder. Colloidal silicon dioxide and magnesium stearate were employed as flow promoter and lubricant, respectively.

3.5.1 Effect of microcrystalline cellulose (MCC) to mannitol ratio

The effect of MCC to mannitol ratio on the characteristics of orally disintegrating tablet is presented in Table 3.2. As can be seen in the table, there is a significant decrease ($p < 0.05$) in disintegration time as well as wetting time as the MCC to mannitol ratio decreases from 2:1 to 1:3. Disintegration time was shorter in those formulations that contain high proportion of mannitol. This might be due the good aqueous solubility, negative heats of solution and good wetting properties of mannitol, which results in improved water uptake, thereby decreasing disintegration time. Other studies also showed the same effect (Singh and Singh, 2009; Lokesh *et al*.,2012) .On the contrary, those formulations containing higher ratio of MCC showed good tablet integrity with a significant increase in tablet hardness and a significant decrease in friability (Table 3.2).

This is in agreement with the reports of Ratnaparkhi *et al* (2012), which suggests that an increase in MCC to mannitol ratio leads to a significant increase in tablet hardness. Formation of strong hydrogen bonding between hydroxyl groups due to presence of large number of free hydroxyl groups and thus interaction force at contact points between particles may be the reason for the increased hardness. As a result, MCC to mannitol ratio of 2:1 and 1:1 were selected for further studies.

Table 3. 2: Physicochemical properties of plane phenobarbitone ODTs.

Formulation	disintegration time (sec.) ± SD	Friability (%)	Hardness (N) ± SD	Wetting Time (sec. ± SD)
F1	58.55 ±0.95	0.282	132.2±1.48	93.18±1.41
F2	51.70±1.03	0.302	119.6±1.84	86.53±1.98
F3	68.17±1.33	0.318	114.0±1.89	94.81±1.27
F4	33.83 ±1.47	0.568	99.0 ± 2.54	51.07± 0.96
F5	37.17 ±2.99	0.610	91.2 ±3.16	55.51±1.71
F6	40.67 ±1.97	0.718	81.4 ±2.32	61.29±2.22
F7	25.83 ±0.75	1.107	67.0 ±1.94	40.85±1.57
F8	28.83 ±0.98	1.213	63.8 ±2.39	43.20±1.22
F9	31.83 ±0.75	1.395	58.4 ±2.27	48.53±1.27
F10	21.17 ± 0.98	1.716	47.8 ±3.33	33.76±1.35
F11	24.83 ±1.03	2.059	42.0 ±2.31	40.48±1.45

F1-F11: formulation of plain orally disintegrating phenobarbitone tablets

3.5.2 Effect of SSG concentration

Sodium starch glycolate (Explotab) is a cross-linked low substituted carboxymethyl ether of poly-α-glucopyranose, in which about 15% of the glucose units are carboxymethylated. It is obtained by a suitable treatment of potato starch and possesses a particle size in the range of 35–55 µm (Perioli *et al.*, 2012). It swells to 7-12 folds in a three dimensions within 30 Sec. The main mechanism is water uptake followed by rapid and enormous swelling (El-Nabarawi *et al.*, 2013).

In order to investigate the effect of SSG concentration on tablet properties, various formulations containing 4, 8, and 12% were prepared (Table 2.1). As can be seen in Table 3.2, as the concentration of SSG increases the disintegration time also increased significantly, except in case of MCC to Mannitol ratio of 2:1 where the disintegration time first decreased significantly ($p<0.05$) when concentration of SSG increased from 4 to 8 %, and then increased significantly when the concentration of SSG increased from 8 to 12%. The same effect was observed by (Prajapati and Patel, 2010). In addition, increasing the level of SSG increased tablet friability and decreased hardness. These findings are in agreement with the work reported by (Indhumathi and Rajashekar, 2013). Thus, the levels of SSG for further studies were kept at 4 and 8%.

3.5.3 Effect of compression force

In order to investigate the effect of compression force on various tablet characteristics, tablets were compressed at 12 KN, 17 KN and 20 KN while keeping other factors constant (F4). As can be expected, increasing compression force resulted in a significant decrease in friability ($p=0.0012$) and significant increase in disintegration time ($p<0.0001$), Table 3.3. In addition, there is a significant increase ($p < 0.05$) in hardness and wetting time as compression force was increased from 12 to 20 KN.

Table 3. 3: Effect of compression force on characteristics of phenobarbitone ODTs.

Formulation	Compression force (KN)	Disintegration time (Sec.± SD)	friability %	Wetting time (sec.± SD)	Hardness (N ± SD
F a 4	12	27.53 ± 1.00	0.28	50.33 ± 2.52	73.33 ± 3.06
F b 4	17	57.78 ± 1.09	0.19	78.67 ±2.08	93.00 ± 2.65
F c 4	20	70.26 ± 1.61	0.14	95.33 ± 1.53	106.67 ±2.08

3.6 Formulation optimization

Screening the important factors, among a wider set of possible controllable factors, is the first exercise during optimization study in RSM. In direct compression of tablet, the various factors considered include compression force, level of superdisintegrant and MCC/mannitol and all of them affected the various responses considered- disintegration time, hardness and wetting time. Besides, in the process of microsphere formation, the polymer to drug ratio was found to have a very significant effect on drug release. Hence, those four factors were further optimized.

Consequently, to deal with the aforementioned factors fractional factorial design was chosen. Accordingly, the 8 formulations of taste masked orally disintegrating phenobarbitone a tablet with the selected levels of factors are summarized in Table 3.4.

The optimization of the responses was conducted with the help of Design-Expert® 8.0.7.1 software, which was used to construct the response surface and contour plots for the fitted polynomial equations and to find the optimum area, at which the desired responses could be achieved.

Table 3. 4: Level of fractional factorial design and formulations of taste-masked orally disintegrating phenobarbitone tablets studied.

Formulation	compression force, A (KN)	SSG, B (%)	MCC:mannitol, C (W/W)	polymer:drug, D (W/W)
OF1	17.0 (+1)	4.0 (-1)	1.0 (-1)	3.0 (+1)
OF2	10.0 (-1)	4.0 (-1)	1.0 (-1)	1.0 (-1)
OF3	10.0 (-1)	8.0 (+1)	1.0 (-1)	3.0 (+1)
OF4	10.0 (-1)	4.0 (-1)	2.0 (+1)	3.0 (+1)
OF5	17.0 (+1)	8.0 (+1)	2.0 (+1)	3.0 (+1)
OF6	10.0 (-1)	8.0 (+1)	2.0 (+1)	1.0 (-1)
OF7	17.0 (+1)	8.0 (+1)	1.0 (-1)	1.0 (-1)
OF8	17.0 (+1)	4.0 (-1)	2.0 (+1)	1.0 (-1)

3.6.1 Characteristics of microsphere containing mixtures

Powder flow properties are crucial in handling and processing operations such as flow from hoppers, mixing and compression. A uniform flow from the hopper into the die cavity ensures uniform tablet weight and drug content. Poor flowing powders present many difficulties to the pharmaceutical industry (El-Nabarawi *et al*., 2013). Accordingly, the bulk density, tapped density, Hausner's ratio, Carr's index and angle of repose of the optimization formulations were determined, Table 3.5. The results of bulk and tapped densities ranged from 0.52 to 0.57 and 0.59 to 0.64, respectively. Whereas the Carr's index and Hausner ratio of all the formulations were below 14.44 and 1.17, respectively, indicating that all the blends have good flow property USP30/NF25 (2007). The angle of repose values of all the formulations were also in the range of 30.18 to 32.10, which also shows the good flowing nature of the blend. The corresponding flow rates of the formulations are also given.

Table 3. 5: Characteristics of microspheres containing powder mixtures of optimization formulations of taste-masked phenobarbitone ODTs.

Formulation	Flow rate (g/s) ± SD	Angle of repose (degree) ± SD	Bulk density (g/ml) ± SD	Tapped density (g/ml) ± SD	Hausner ratio ±SD	Carr's index % ± SD
OF1	4.64±0.29	30.39± 1.29	0.54± 0.02	0.60± 0.03	1.12± 0.02	10.48± 1.57
OF 2	4.69±0.29	31.23± 2.01	0.55± 0.01	0.61± 0.02	1.11± 0.04	9.82± 3.29
OF 3	5.01±0.41	30.19 ± 2.30	0.57± 0.03	0.64± 0.01	1.12± 0.04	11.01± 3.16
OF 4	5.07±0.40	30.18± 1.13	0.56± 0.03	0.63± 0.05	1.12± 0.05	10.94± 3.80
OF 5	3.71±0.63	31.62± 2.16	0.52± 0.05	0.61± 0.05	1.17± 0.02	14.44± 1.48
OF 6	3.77±0.29	30.52± 1.09	0.52± 0.03	0.59± 0.04	1.15± 0.01	12.89± 0.83
OF 7	4.07±0.47	32.10± 2.42	0.53± 0.04	0.61± 0.02	1.15± 0.05	13.08± 3.57
OF 8	4.93±0.24	30.25± 2.32	0.57± 0.02	0.64± 0.02	1.12± 0.01	10.82± 0.90

OF- Optimization Formulation

3.6.2 Evaluation of tablet properties

The various physicochemical properties of the taste masked phenobarbitone ODTs optimization formulations are given in Table 3.6. Tablets require a certain amount of strength or hardness to withstand the mechanical shocks of handling in manufacturing, packaging as well as in shipping. Besides, tablet hardness is closely linked to disintegration time. For conventional compressed tablet, a hardness of 50 N is the minimum requirement for satisfactory tablet product. As can be seen in the table, the hardness of the tablets was found to be in the range of 41.3 ± 0.82 N to 135.9 ± 1.73 N. Therefore, except OF2, all the tablets have acceptable hardness. Another measure of tablet strength is friability. Conventional compressed tablets that lose less than 1% of their weight are generally considered acceptable. The percentage friability for all the formulations was below 0.5%, indicating that the friability is within the acceptable limit USP30/NF25 (2007)

For orally disintegrating tablets, disintegration time is considered to be one of the important criteria in selecting the best formulation (Reddy *et al.*, 2014). As shown in Table 3.6, the *in vitro* disintegration time ranged from 21±1.59 - 90±2.74 sec. Formulation (OF2), which contains 4% supperdisintegrant, 1:1 MCC/manitol, 1:1polymer/ drug ratio and compressed at 10 KN has the shortest disintegration time (21± 1.59 sec.). Whereas, formulation (OF5), containing the highest level of all the four factors exhibited the longest disintegration time (90±2.74 sec.).

The dissolution process of a tablet depends upon wetting followed by disintegration of the tablet. Hence wetting time may be used as another confirmative test for the evaluation of fast dissolving tablets (Rao and Kulkarni, 2010).Wetting time was determined for all the eight formulations (Table3.6), and the results revealed that the wetting time ranged from 30±2.11 - 170±3.27 sec.

Table 3. 6: Physicochemical properties of tablets prepared for optimization of taste masked phenobarbitone ODTs.

Formulation	Disintegration time(sec) ± sd	Wetting time(sec) ± sd	Hardness(N) ± sd	Friability (% ± sd)
OF1	75± 2.2	118± 2.6	107.0 ± 2.71	0.13± 0.012
OF 2	21± 1.6	30± 2.1	41.3 ± 0.82	0.32± 0.021
OF 3	40± 1.6	48± 2.7	51.2 ± 1.03	0.26± 0.015
OF 4	25± 1.5	44± 2.4	72.8 ± 1.62	0.19± 0.010
OF 5	90± 2.7	170± 3.3	135.9 ± 1.73	0.10± 0.007
OF 6	36 ± 1.4	43± 1.8	61.3 ± 1.77	0.24± 0.013
OF 7	71± 2.7	80± 3.3	71.2 ± 2.35	0.20± 0.014
OF 8	64± 1.4	125± 2.7	101.2 ± 2.35	0.15± 0.010

The *in vitro* drug release behaviors of the different formulations are shown in Figure 3.3. The USP30/NF25 (2007) specification for the release of phenobarbital from tablets of phenobarbital states that" not less than 75% of the label claim of phenobarbital should be dissolved in 45 min". As can be seen in the figure, all the formulations released more than 75% of the label claim within 20 min. Thus, all the formulation passed the USP requirement.

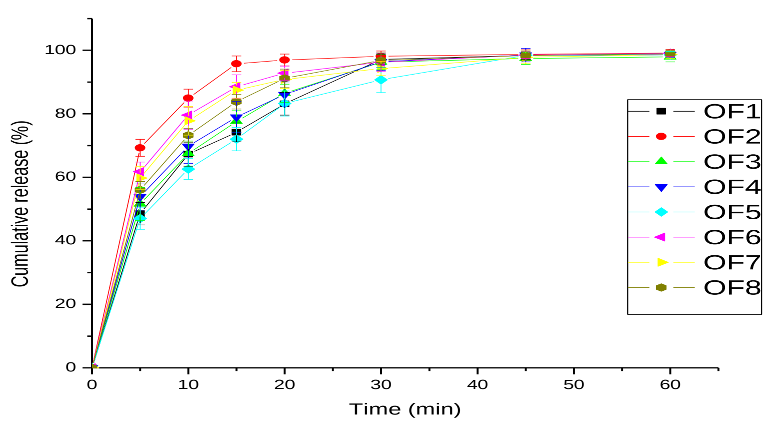


Figure 3. 3: In vitro dissolution profiles of different optimization formulations of taste-masked phenobarbitone ODTs.

3.6.3 Selection and checking adequacy of mathematical model

Model summary statistics for the selected significant models are shown in Table 3.7. It can be observed that R^2 is high for all responses (DT time, wetting time, hardness, % release in 5 min and % release in 15min), which suggest a high degree of correlation between the experimental and predicted responses. In addition, the predicted R^2 value is in good agreement with the adjusted R^2 value, resulting in reliable models. It is necessary to check the fitted model to ensure that it provides an adequate approximation to the real system. ANOVA table has been used to summarize the test for significance of regression model as well as for individual model coefficient (Kohli and Singh, 2011 , Bose *et al.*, 2013).The larger the value of F and the smaller the value of p, the more significant is the corresponding coefficient term. The value of p was lower than 0.05, indicating that the model may be considered to be statistically significant (Liu *et al.*, 2010). ANOVA of the responses indicated that response surface models developed for all responses were significant and adequate. Influence of formulation variables and process variable on the response are shown in Tables 3.8-3.16

Table 3. 7: Numerical test results for selection of response models of DT time, wetting time, hardness, % release within 5 min and % release within 15 min.

Response	Source	R-Squared	Adjusted R-Squared	Predicted R-Squared	Adequate precision
DT time	R2FI	0.9947	0.9877	0.9626	29.963
Wetting time	R2FI	0.9952	0.9833	0.9237	23.805
Hardness	R2FI	0.9999	0.9995	0.9976	160.259
% release in 5 min	RMain effects	0.8892	0.8449	0.7163	9.932
% release in 15 min	RMain effects	0.9254	0.8955	0.8089	11.127

The best fitting mathematical model was selected based on the comparisons of several statistical parameters, including multiple correlation coefficient (R^2) and adjusted multiple correlation coefficient (adjusted R^2) , and the predicted residual sum of square (PRESS), provided by Design-Expert®8.0.7.1 Software. Consequently, the selected model for drug disintegration time is Reduced2FI. The goodness of fit of the model was checked by determination coefficient (R^2) of 0.9947. In this case, the R^2 value indicates that 99.47% of the variability in response could be explained and only 0.53% of the total variation cannot be explained by the model. The "Pred R-Squared" of 0.9626 is in reasonable agreement with the "Adjusted R-Squared" of 0.9877. The aforementioned results indicate that the Reduced 2FI model provided an excellent explanation for the relationship between the independent variables and the corresponding response.

Similarly, the selected model for wetting time and hardness is Reduced 2FI. The determination coefficient (R^2) for wetting time and hardness are 0.9952 and 0.9999, respectively. So, R^2 values of both responses are closer to 1, which indicates a better prediction of the models, for a good fit of a model. The wetting time predicted R^2 of 0.9237 is in reasonable agreement with the adjusted R^2 of 0.9833. Correspondingly, hardness “Predicted R^2” of 0.9976 is in reasonable agreement with the “Adjusted R^2” of 0.9995. Furthermore, the Reduced 2FI had maximum “Predicted R^2”and “Adjusted R^2” values.

Conversely, the selected model for % drug release in 5 min and 15 min is Reduced Main effects. The determination coefficient (R^2) for % drug release in 5 min is 0.8892 which indicates 88.92% of the variability in the response could be explained by the model. Even though, “Predicted R^2”and “Adjusted R^2” values are smaller than other response values, the "Pred R-Squared" of 0.7163 is in reasonable agreement with the "Adj R-Squared" of 0.8449. But, the determination coefficient (R^2) for % drug release in 15 min is 0.9254 which is slighty greater than that of % drug release in 5 min. So, the R^2 value indicates that 92.54% of the variability in response could be explained and only 7.46% of the total variation cannot be explained by the model. Moreover, the "Pred R-Squared" of 0.8089 is in reasonable agreement with the "Adj R-Squared" of 0.8955.

As shown in Table 3.8, the Reduced 2FI model is statistically significant for disintegration time ($P < 0.05$), which is desirable as it indicates that the terms in the model have significant effect on the response.

As depicted in Table 3.8, A (compression force), B (conc.of SSG), and C (polymer to drug ratio), are significant model terms where as AD (interaction effect of compression force and polymer to drug ratio) is insignificant model terms. The Model F-value of 141.86 implies the model is significant. There is only a 0.10% chance that a "Model F-Value" this large could occur due to noise. However, as shown in Table 3.9, further reduced the Model F-value of 70.67 implies the model is significant. There is only a 0.06% chance that a "Model F-Value" this large could occur due to noise.

Table 3. 8**:** Summary of ANOVA results of response surface Reduced 2FI model for disintegration time.

Source	Sum of Squares	df	Mean square	F-value	p-value	Remark
Model	4539.50	4	1134.88	141.86	0.0010	significant
A-CF	3960.50	1	3960.50	490.06	0.0002	significant
B- SSG	338.00	1	338.00	42.25	0.0074	significant
D-polymer:drug	180.50	1	180.50	22.56	0.0177	significant
AD	60.5	1	60.5	7.56	0.707	Not significant
Residual	24	3	8.00			
Cor Total	4563.5	7				

Table 3. 9: Summary of ANOVA results of response surface back eliminated Reduced 2FI model for disintegration time.

Source	Sum of Squares	df	Mean square	F-value	p-value	Remark
Model	4479.00	3	1493.00	70.67	0.0006	significant
A-CF	3960.50	1	3960.50	187.48	0.0002	significant
B-SSG	338.00	1	338.00	16.00	0.0161	significant
D-polymer:drug	180.50	1	180.50	8.54	0.0431	significant
Residual	84.50	4	21.13			
Cor Total	4563.50	7				

For wetting time, the selected model is Reduced 2FI with F-value of 83.47 and p-value of 0.0119 as shown in Table 3.10, which implies the model is significant. The greater the F-value is from unity, the more certain it is that the factors explain adequately the variation in the data about its mean, and the estimated factor effects are real (Bari *et al*., 2010). There is only a 1.19% chance that a "model F-value" this large could occur due to noise. The P-values less than 0.05 indicate model terms are significant. In this case, A (compression force), C (MCC/mannitol), D (polymer to drug ratio) and AC (interaction effect of compression force and MCC/mannitol are significant model terms. However, AD (interaction effect of compression force and polymer to drug ratio) (p=0.0735) is insignificant model term.

Table 3. 10: Summary of ANOVA results of surface response Reduced 2FI model for wetting time.

Source	Sum of Squares	df	Mean square	F-value	p-value	Remark
Model	17633.00	5	3526.60	83.47	0.0119	significant
A-CF	13448.00	1	13448.00	318.30	0.0031	Significant
C-MCC/mannitol	1404.50	1	1404.50	33.24	0.0288	Significant
D-polymer/drug	1300.50	1	1300.50	30.78	0.0310	Significant
AC	968.00	1	968.00	22.91	0.0410	Significant
AD	512.00	1	512.00	12.12	0.0735	Not significant
Residual	85.50	2	42.25			
Cor Total	17717.50	7				

Model significance is desirable as it indicates that the terms in the model have significant effect on the response. As shown in Table 3.11, model of the response was significant (p=0.0039 for linear model of hardness). ANOVA results in the table revealed that the main effects of three parameters, A (compression force p=0.0017) C (MCC/mannitol) (p=0.0032) and D (polymer/drug) (p = 0.0035), were significant model terms for the linear model of hardness, whereas B (conc. of superdisintegrant) (p=0.1166) was insignificant model term. Simillaly both interaction terms, AC (interaction effect of compression force and MCC/mannitol) (p=0.0184) and AD (interaction effect of compression force and polymer to drug ratio) (p=0.0065) are significant model terms. But, the main effects of B (conc.of superdisintegrant) (p=0.1166) was insignificant model term.Hence, backward elimination procedure was applied to reduce insignificant terms so as to increase the model's predictive efficiency.\

Table 3. 11: Summary of ANOVA results of surface response Reduced 2FI model for hardness.

Source	Sum of Squares	df	Mean square	F-value	p-value	Remark
Model	7108.87	6	1184.81	37913.96	0.0039	significant
A- CF	4450.96	1	4450.96	1.424E+005	0.0017	significant
B- SSG	0.91	1	0.91	29.16	0.1166	Not significant
C- MCC/mannitol	1262.53	1	1262.53	40401.00	0.0032	significant
D- polymer/drug	1055.70	1	1055.70	33782.44	0.0035	significant
AC	37.41	1	37.41	1197.16	0.0184	significant
AD	301.35	1	301.35	9643.24	0.0065	significant
Residual	0.031	1	0.031			
Cor Total	7108.90	7				

Results in Table 3.12 indicate that the further reduced model was more significant (p=0.0003) than the unreduced model (p=0.0039) indicating the model predictive efficiency might be improved, although in both cases, the model was significant. Moreover, the significance of model terms A (compression force, p=0.0001); C (MCC/mannitol, p=0.004); D (polymer/drug, p=0.004); AC (interaction effect of compression force and MCC/mannitol, p=0.0124); and AD (interaction effect of compression force and polymer to drug ratio, p=0.0016) also further improved.

Table 3. 12: Summary of ANOVA results of surface back eliminated response Reduced 2FI model for hardness.

Source	Sum of Squares	df	Mean square	F-value	p-value	Remark
Model	7107.96	5	1421.59	3016.64	0.0003	significant
A- CF	4450.96	1	4450.96	9445.01	0.0001	significant
C-MCC/mannitol	1262.53	1	1262.53	2676.11	0.0004	significant
D-polymer/drug	1055.70	1	1055.70	2240.21	0.0004	significant
AC	37.41	1	37.41	79.39	0.0124	significant
AD	301.35	1	301.35	637.47	0.0016	significant
Residual	0.94	2	0.47			
Cor Total	7108.90	7				

ANOVA of percent drug released within 5 min showed that the F value of 19.74 implies significant model (Table 3.13). There is only 0.73% chance that the 'model F-value' this large could occur due to noise. Values of p-value less than 0.0500 indicate model terms are significant. In this case, the model terms A (compression force) and D (polymer/drug) are found to be significant, but AB (interaction effect of compression force and conc. of superdisintegrant) is insignificant model term. Therefore, backward elimination procedure was applied to reduce insignificant term so as to increase the model's predictive efficiency.

Table 3. 13: Summary of ANOVA results of surface response Reduced 2FI model for percent drug released within 5 min.

Source	Sum of Squares	df	Mean square	F-value	p-value	Remark
Model	360.01	3	120.00	19.74	0.0073	significant
A-CF	79.19	1	79.19	13.03	0.0226	significant
D-polymer/drug	262.55	1	262.55	43.18	0.0028	significant
AB	18.27	1	18.27	3.01	0.1580	Not significant
Residual	24.32	4	6.08			
Cor Tota	384.33	7				

Results in Table 3.14 indicate that the further reduced model was more significant (F = 20.06, p= 0.0041) than the reduced model (F = 19.74, p=0.0073) indicating the model predictive efficiency might be improved, although in both cases, the model was significant. The higher the F-value, the greater is the significance of the corresponding variable (term) to cause effect. In a similar manner, as shown in Table 3.14, the effects of all the model terms in the further reduced model become more significant on the response as compared to their respective effects on the response in the unreduced model.

Table 3. 14: Summary of ANOVA results of surface response back eliminated Reduced 2FI model for percent drug released within 5 min.

Source	Sum of Squares	df	Mean square	F-value	p-value	Remark
Model	341.74	2	170.87	20.06	0.0041	significant
A-CF	79.19	1	79.19	9.30	0.0285	significant
D-polymer/drug	262.55	1	262.55	30.82	0.0026	significant
Residual	42.59	5	8.52			
Cor Tota	384.33	7				

For percent drug released within15 min, the selected model is reduced main effect with F-value of 31.00 and p-value of 0.0015 as shown in Table 3.15 which implies the model is significant. The only significant model term for percent drug released within 15 min is polymer/drug with p-value 0.0006 while compression force is insiginificant with p-value 0.0983. Hence, backward elimination procedure was applied to reduce insignificant term so as to increase the model's predictive efficiency. As a result model predictive efficiency was improved.

Table 3. 15:Summary of ANOVA results of surface response Reduced main effect model for percent drug released within 15 min.

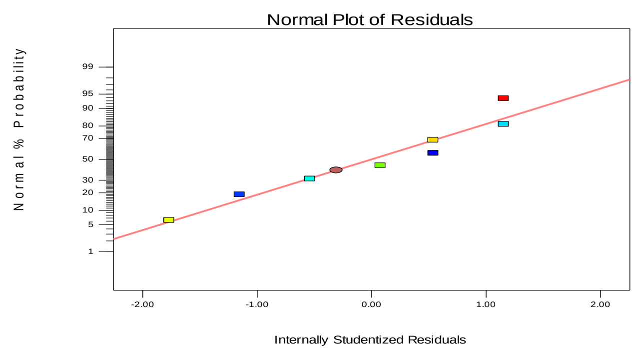
Source	Sum of Squares	df	Mean square	F-value	p-value	Remark
Model	485.05	2	242.53	31.00	0.0015	significant
A-CF	32.20	1	32.20	4.12	0.0983	Not significant
D-polymer/drug	452.85	1	452.85	57.88	0.0006	significant
Residual	39.12	5	7.82			
Cor Tota	524.18	7				

Results in Table 3.16 indicate that the further reduced model was more significant (F = 38.10, p=0.0008) than the reduced model (F = 31.00, p= 0.0015) indicating the model predictive efficiency might be improved, although in both cases, the model was significant. The higher the F-value, the greater is the significance of the corresponding variable (term) to cause effect.

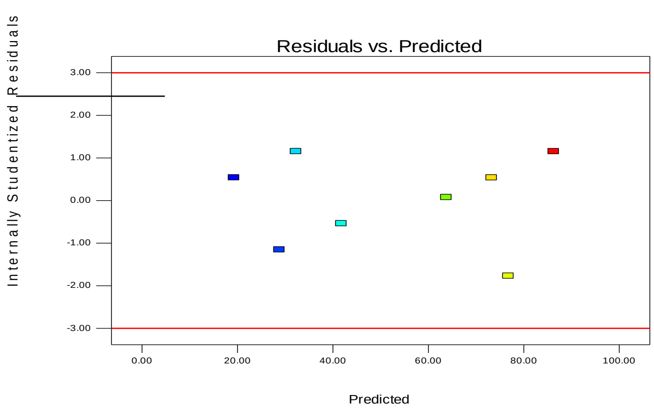
Table 3. 16: Summary of ANOVA results of surface response further Reduced main effect model for percent drug released within 15 min.

Source	Sum of Squares	df	Mean square	F-value	p-value	Remark
Model	452.85	1	452.85	38.10	0.0008	significant
D-polymer/drug	452.85	1	452.85	38.10	0.0008	significant
Residual	71.32	6	11.89			
Cor Tota	524.18	7				

The normal probability plots of the residuals and the plots of the residuals versus the predicted response for disintegration time, wetting time, hardness, % drug release within 5 min and % drug release within 15 min are shown in Figures 3.4 - 3.8. The important information on the model performance is summarized in residuals (i.e. difference between observed and predicted values) providing a clear view for any discrepancy in fit to the model. Hence, two plots related to residuals: the normal probability plot of residuals and the plot of internally studentized residuals versus predicted values are considered as additional tests of model adequacy checking tools (Bari *et al*., 2010). A check on the plots in Figure 3.4 A, 3.5 A, 3.6 A, 3.7 A and 3.8A show that points or point clusters are placed closely to the diagonal line implying that the errors are distributed normally for all responses. Figures 3.4 B, 3.5 B, 3.6 B, 3.7 B and 3.8 B indicate that the points are randomly scattered, with no obvious pattern or structure, and all values lie within the recommended range of −3 and +3 outlier detection limits.

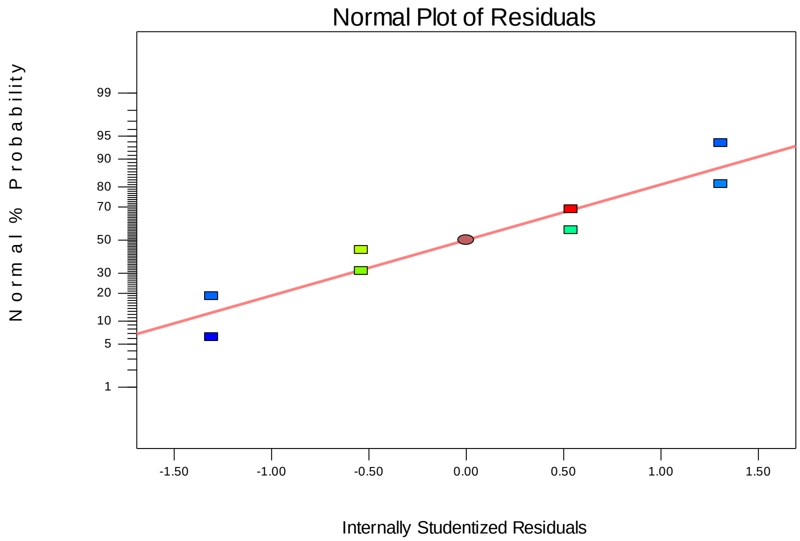


A

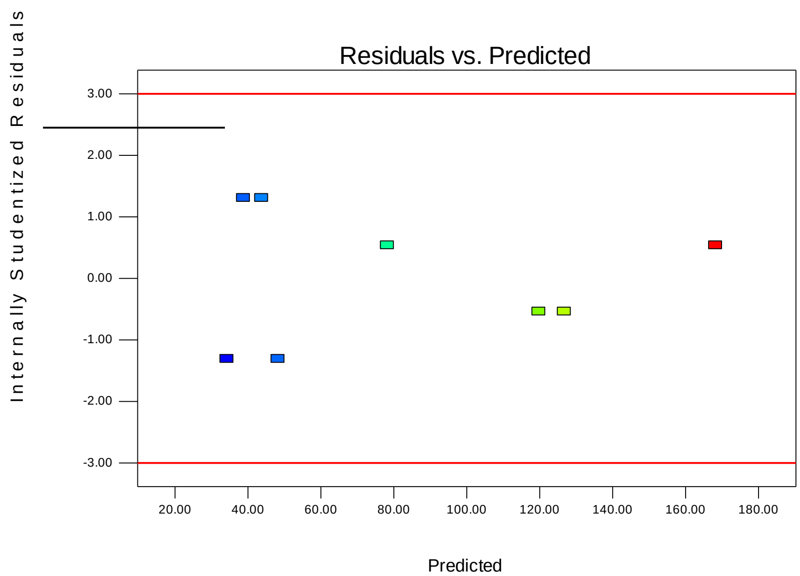


B

Figure 3. 4: A: Normal probability plot of residuals, B: Plots of the residuals against predicted response for disintegration time.

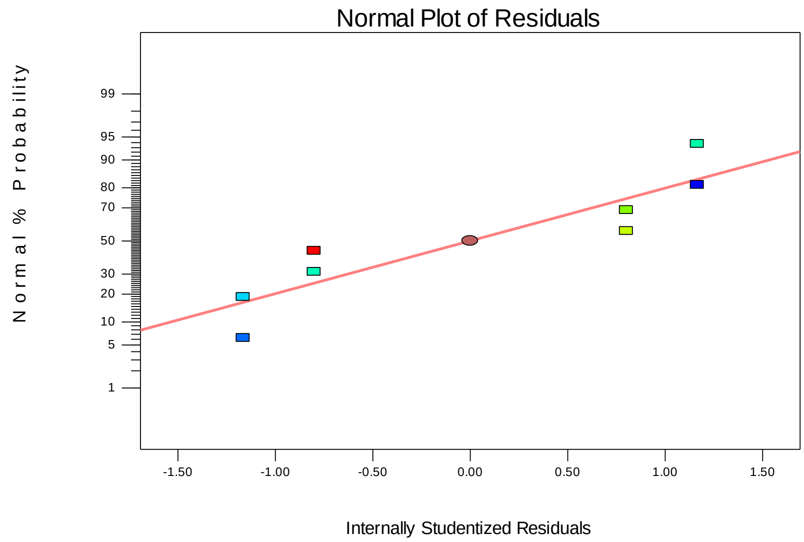


A

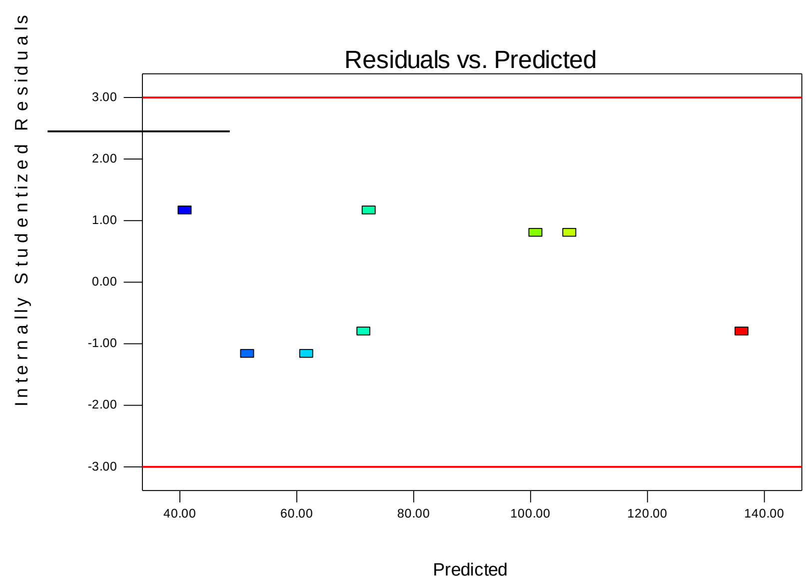


B

Figure 3. 5: A: Normal probability plot of residuals, B: Plots of the residuals against predicted response for wetting time.

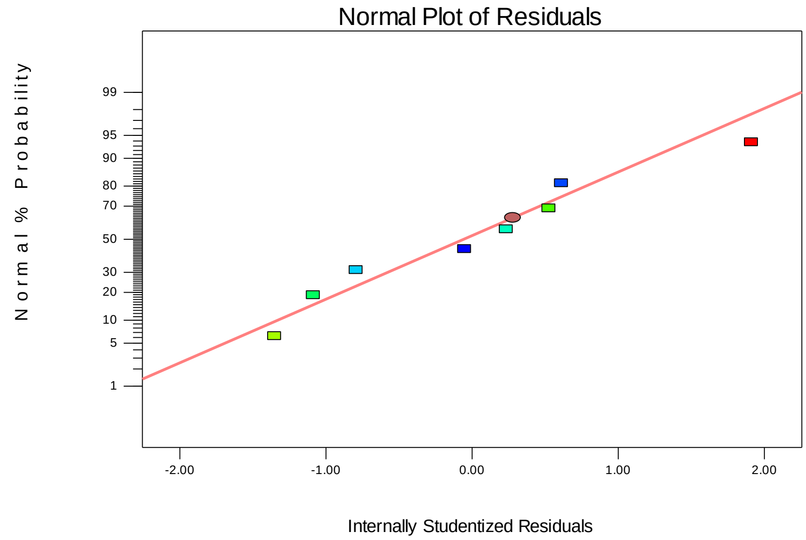


A

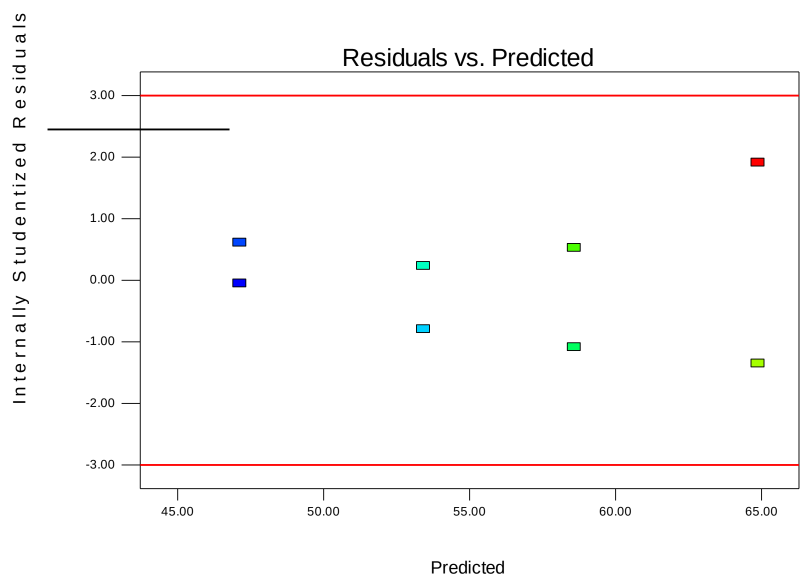


B

Figure 3. 6: A: Normal probability plot of residuals, B: Plots of the residuals against predicted response for hardness.

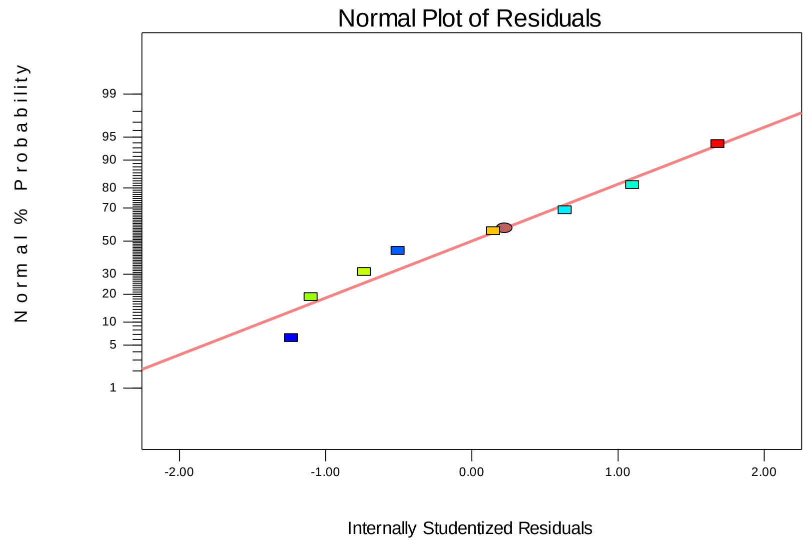


A

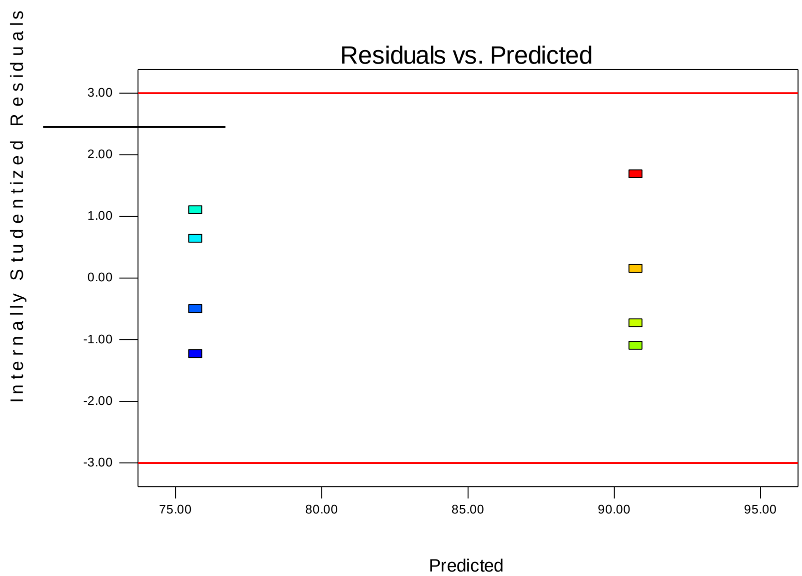


B

Figure3. 7: A: Normal probability plot of residuals, B: Plots of the residuals against predicted response for % drug release within 5 min.



A



B

Figure 3. 8: A: Normal probability plot of residuals, B: Plots of the residuals against predicted response for % drug release within 15 min.

It can be concluded from these analyses that the proposed models are adequate and there is no reason to infer any violation of the independence or constant variance assumption (Bari *et al.*, 2010). Therefore, the models were used for further analysis.

3.6.4 The mathematical regression models

The final mathematical regression models in terms of coded factors (Eq. 3.1 to Eq. 3.5) were developed using model term coefficients.

Disintegration time (Y1) = + 52.75 + 22.25* A+ 6.5*B +4.75*D+2.75 *A*D Eq. 3.1

Wetting time (Y2) = +82.25+41.00*A+13.25*C+12.75*D+11.00*A*C+8.00*A*D Eq. 3.2

Hardness (Y3) =+80.24+23.59*A +12.56*C +11.49*D +2.16*A*C +6.14*A*D Eq. 3.3

Drug release after 5 min (Y4) =+56.00 - 3.15 * A - 5.73* D Eq. 3.4

Drug released after 15 min (Y5) =+83.22- 7.52 * D Eq. 3.5

Where, A is compression force, B is concentration of superdisintegrant, C is MCC to mannitol ratio, and D is polymer to drug ratio.

Coefficients of the developed models have physical meanings on the response variables. A coefficient is the amount the response changes when that term is changed by one unit, while holding the other terms constant. Both the magnitude and sign of coefficients are important: the magnitude implies the strength, whereas, the sign indicates the direction of that factor variable on the corresponding response variable. A positive sign indicates a positive effect where as a negative sign indicates a negative effect on the response (Nutan *et al.*, 2005; Ranjan *et al.*, 2012).Thus, the models were accordingly analyzed.

As evidenced by Eq. 3.1, three parameters (compression force (A), concentration of superdisintegrant (B) and polymer to drug ratio (D) significantly affect disintegration time positively; however, the effect of compression force was the strongest with higher coefficient (22.25) than the other variables investigated.

Besides, on the contrary to what is expected, an increase in concentration of the superdisintegrant leads to increase in the disintegration time of the orally disintegrating tablet. The reason behind this increase may be attributed to formation of a viscous gel layer on the surface of the tablet, which prevents further penetration of the disintegration medium and hinder the disintegration. This prevents the penetration of water to the core of the tablet (Nasser *et al.*, 2012).

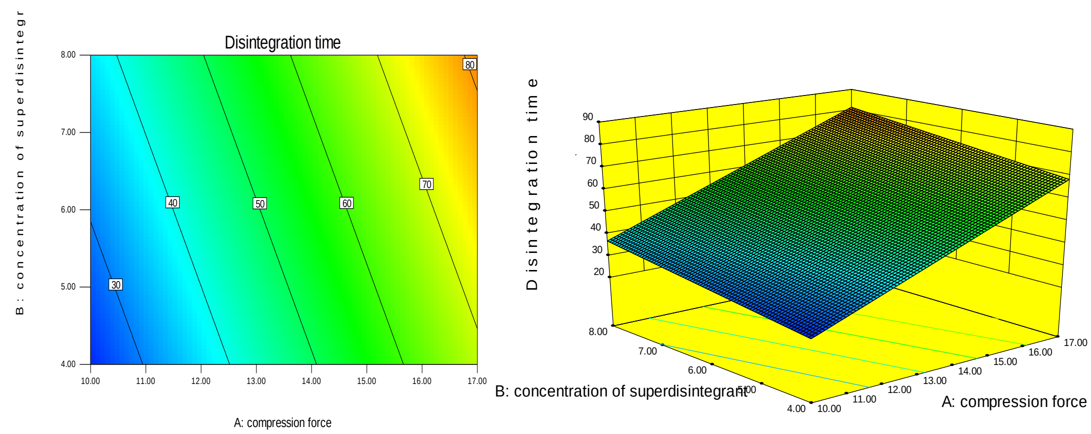
From Eqs. 3.2 and 3.3, it can be observed that compression force (A), MCC to mannitol ratio (C), polymer to drug ratio (D), interaction terms (AC and AD) have a significant positive effect on tablet wetting time and hardness. Therefore, increasing these factors results in an increase in tablet hardness and wetting time. Again in these cases, the effect of compression force was found to be the more determinant than the others on both wetting time and hardness as it had higher coefficient, +41.00 and +23.59, respectively.

On the contrary, as can be seen from Eq. 3.4, the parameters compression force (A) and polymer to drug ratio (D) affect % drug release after 5 min. negatively. Besides, the effect of polymer to drug ratio was more significant than the effect of compression force. Similarly, as can be observed from Eq. 3.5, the polymer to drug ratio has a negative effect on % drug release after 15 min. indicating that increasing the polymer concentration decreases drug release rate. Compression force had no significant effect at the later stage, which might be attributed to the rapid disintegration of the tablets. Due to fast and complete release of the drug from the formulation, drug release was studied only at two points, where the formulations show great difference in their release profile.

3.6.5 Contour plot and surface response analysis

The three-dimensional response surface plots, obtained as a function of two factors maintaining all other factors constant, are helpful in understanding the effects of each factor as well as their interaction (Pandya *et al.*, 2011). The corresponding contour plots, represented by the projection of the response surfaces in the x–y plane, provides a straight forward determination of the effects of the independent variables on the dependent variables (Nutan *et al.*, 2005; Liu *et al.*, 2010). In all the presented figures, two factors were selected according to their significance and the third and fourth factors were kept at level zero (that is mid-point).

The contour and response surface plots shown in Figs 3.9 A and B, respectively, indicate the combined effect of compression force and concentration of supperdisintegrant on disintegration time. The diagonal parallel straight lines of the contour plot and the non-twisted response surface indicate that there was no interaction effect of the two parameters on the disintegration time. However, the plots show that the linear model components individually affect the disintegration time significantly, with comparatively more significant effect of compression force than the concentration of supperdisintegrant.

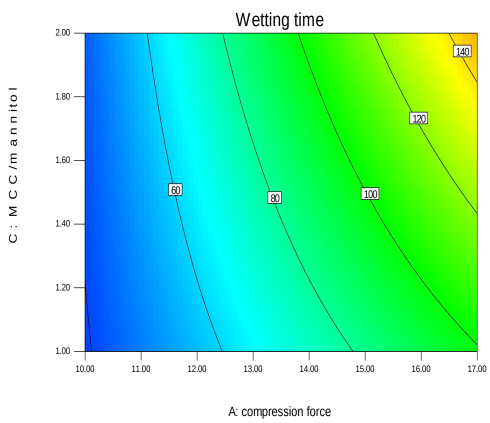


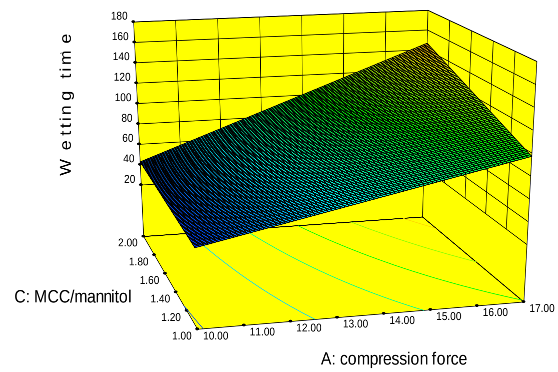
A B

Figure 3. 9: A: Contour B: Surface response plot of disintegration time as a function of compression force and concentration of superdisintegrant.

According to Figures 3.9 A and B, the most suitable conditions for minimum disintegration time within the investigated ranges were found to be at the lowest compression force (coded A = -1) and at the lowest used concentration of superdisintegrant (coded B = -1) keeping other two factors at mid-point.

The combined effect of compression force and MCC to mannitol ratio on wetting time of the orally disintegrating tablet is shown in Figs 3.10 A and B. The plots indicate that both compression force and MCC to mannitol ratio play a very significant role in influencing the response wetting time. However, the effect of compression force appeared to be more pronounced as compared to the MCC to mannitol ratio. This was confirmed from the coefficients in the mathematical model generated for wetting time (Eq. 3.2). Moreover, as the curvilinear contours of Figures 3.10 A indicate, the interactive effect of the two variables was significant.

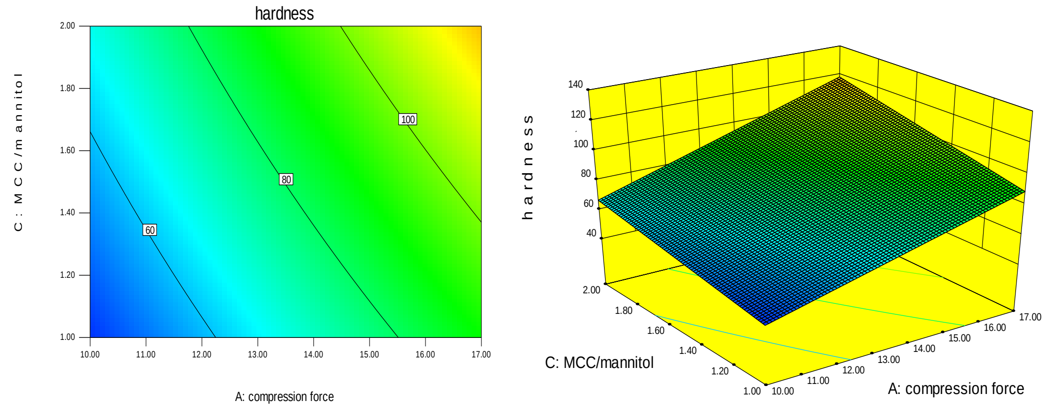




A B

Figure 3. 10: A: Contour, B: surface plot of wetting time as a function of compression force and MCC to mannitol ratio.

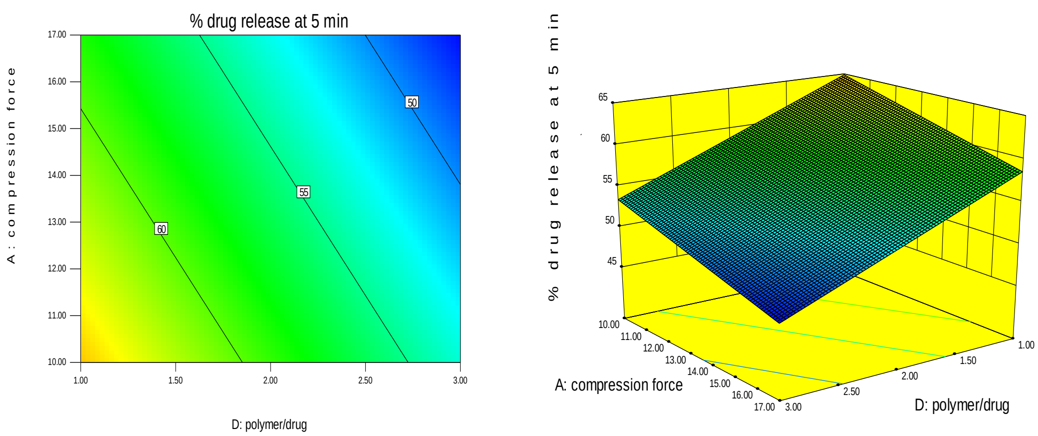
Figures 3.11 A and B depict the combined effect of compression force and MCC to mannitol ratio on tablet hardness. The contour plot indicates that both compression force and MCC to mannitol ratio play a very significant role in influencing tablet hardness. In addition, the effect of compression force appeared to be slightly higher as compared to the MCC to mannitol ratio. Besides, the slightly curved diagonal lines on the contour plot show the minor synergistic effect of the variables on the response. This was further confirmed by the coefficients in the mathematical model generated for hardness (Eq.3.3).



A B

Figure 3. 11: A: Contour and B: Surface response plot of hardness as a function of compression force and MCC to mannitol ratio.

Figures 3.12 A and B depict the 2D and 3D representation of the effect of the compression force and polymer to drug ratio on the percent drug release after 5 min. The series of diagonal parallel straight lines in Figure 3.12 A of the contour plot and the non-twisted response surface in Fig 3.12 B indicate that there is no interaction between compression force and polymer to drug ratio on % drug release within 5 min. Both factor showed a negative effect on percent drug release. Besides, the effect of polymer to drug ratio is more pronounced than the effect of compression force.



A B

Figure 3. 12: A: Contour B: Surface response plot of percent drug release within 5 min as a function of compression force and polymer to drug ratio.

The combined effect of compression force and polymer to drug ratio on % drug release within 15 min is shown in Figure 3.13 A and B. The series of vertical parallel straight lines in Figure 3.13 A of the contour plot and the non-twisted response surface in Fig 3.13 B show that compression force has no effect on % drug release within 15 min. Thus, the only factor which significantly affects % drug release within 15 min was polymer to drug ratio.

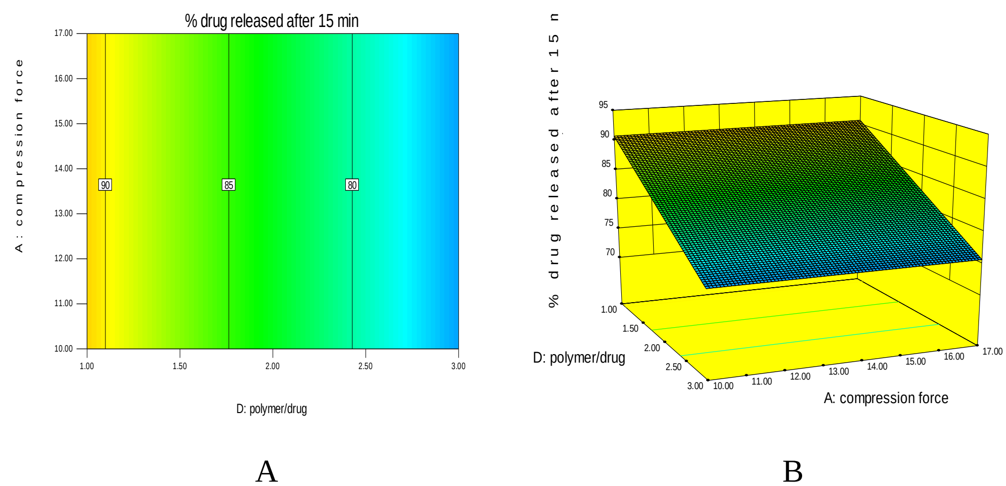


Figure 3.13: A: Contour B Surface response plot of % drug release within15 min as a function of compression force and polymer to drug ratio on.

3.6.6 Simultaneous optimization of the response variables

After generating the model polynomial equations to relate the dependent and independent variables, the formulation was optimized for the five responses simultaneously. The final optimal experimental parameters were obtained using both numerical and graphical optimization techniques of Design-Expert® 8.0.7.1software, which allows the compromise among various responses and searches for a combination of factor levels that jointly optimize a set of responses by satisfying the requirements for each response in the set.

3.6.6.1 Numerical optimization

A numerical optimization technique using the desirability approach was employed to develop a new formulation with the desired responses. The optimization was done with constraints for disintegration time, in the range of 0-60 sec. wetting time in the range of 0-60 sec, hardness in the range of 50-80 N, % drug release within 5 min in the range of 50%-100% and % drug release within 15 min in the range of 85-100% as the goals to locate the optimum setting of independent variables in the new formulation as shown in Table 3.17.

Table **3. 17:** Criterion settings of factors and responses for optimization of taste-masked phenobarbitone ODTs.

Variable	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
CF(N)	Range	10	17	1	1	-
SSG (%)	Range	4	8	1	1	-
MCC/Mannitol(W/W)	Range	1	2	1	1	-
Polymer/drug (W/W)	Range	1	3	1	1	-
Disintegration time (sec)	Range	0	60	1	1	5
Wetting time (sec)	Range	0	60	1	1	3
Hardness (N)	Range	50	80	1	1	5
Drug release within 5 min (%)	Range	50	100	1	1	3
Drug release within 15 min (%)	Range	85	100	1	1	5

The desirability function approach is one of the most widely used methods for optimization of multiple response processes. This function searches for a combination of factor levels that jointly optimize a set of responses by satisfying the requirements for each response in the design. Figure 3.14 shows the predicted optimum values and the corresponding levels of parameters according to the set goals. The dot indicates the best solutions found by the Design-Expert solver.

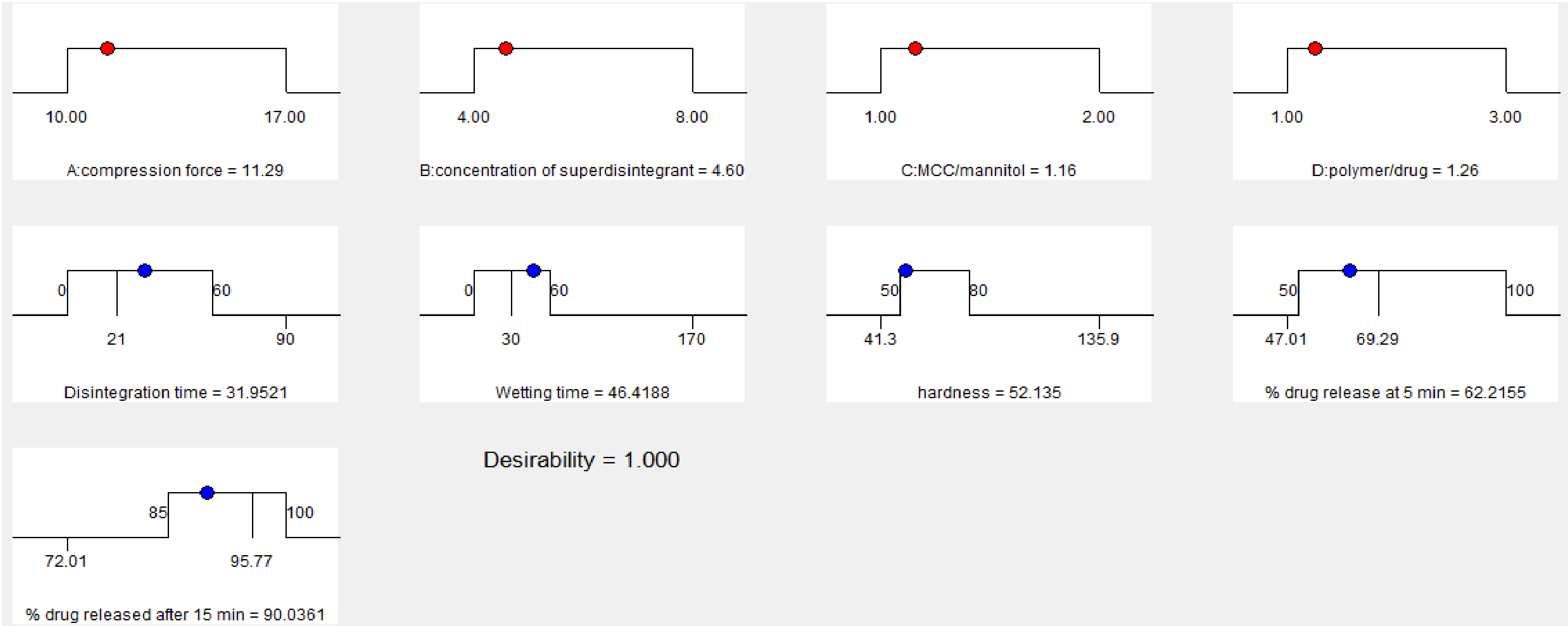


Figure 3.14 : Desirability ramp for numerical optimization of nine goals, namely : compression force, concentration of superdisintegerant, MCC to Mannitol ratio, polymer to drug ratio, disintegration time, wetting time, hardness, % drug release in 5 min and % drug release in 15 min.

In multiple response optimization using desirability approach, individual desirability functions d_i indicate measures of how well the goals for each response are satisfied, whereas overall desirability function is a measure of how well the combined goals for all responses are satisfied. Desirability function ranges from 0 to 1, with value closer to one indicating a higher satisfaction of response goal(s) (Ranjan et al., 2012). In this study, the values of individual desirability functions d_i of disintegration time, wetting time, hardness, drug release within 5 min and drug release within 15 min were obtained from the Design-Expert solver to be 1.00 for all responses, as calculated from the optimal point obtained ($Y_1 = 31.95$, $Y_2 = 46.42$ $Y_3 = 52.13$,$Y_4 = 62.21$, $Y_5 = 90.03$). This indicates that, fully desired responses were achieved for all responses. The overall desirability function (D) was then obtained from the individual desirability functions to be 1.00 from the software solver calculated based on Equation 3.6.

$$D = \left[d_1^{p_1} d_2^{p_2} d_3^{p_3} \ldots d_i^{p_i} \right]^{1/\sum p_i} \qquad \text{Eq.3.6}$$

Where i is the number of responses, d_i the individual desirability functions and p_i is the relative importance of i^{th} response as compared to the others. Importance (p_i) varies from 1 to 5, from least to most important, respectively. Figure 3.15 shows a 3D plot of the overall desirability function D for the (A: compression force and D:polymer to drug ratio) plane.

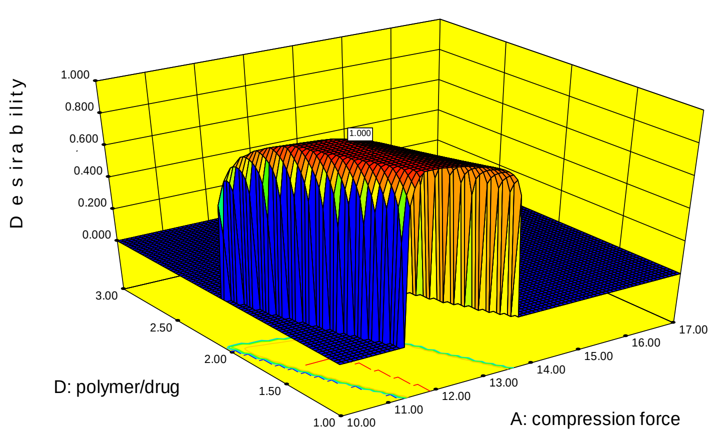


Figure 3.15: 3D view of most desirable operating conditions.

3.6.6.2 Graphical optimization

The graphical optimization allows visual selection of the optimum conditions according to certain criteria. The results of the graphical optimization are the overlay plots. For each response, the lower and/or upper limits have been chosen according to the numerical optimization results. The same criteria proposed in the numerical optimization have been introduced in the graphical optimization.

Figure 3.16 shows the overlay plot in which the yellow area represents the area satisfying the imposed criteria. However, the value of MCC to mannitol and polymer to drug ratio were kept constant at 1.6:1 and 1.26:1, respectively. The point identified by the flag was chosen in the graph as representative of the optimized area corresponding to compression force 11.29 KN, amount of supperdisintegrant of 4.60%, MCC to mannitol 1.6 :1 and polymer to drug ratio of 1.26:1. Under these conditions the model predicts disintegration time of 31.95 sec., wetting time of 46.41, hardness of 52.13, % drug release within 5 min of 62.21% and % drug release within 15 min of 90.03 %.

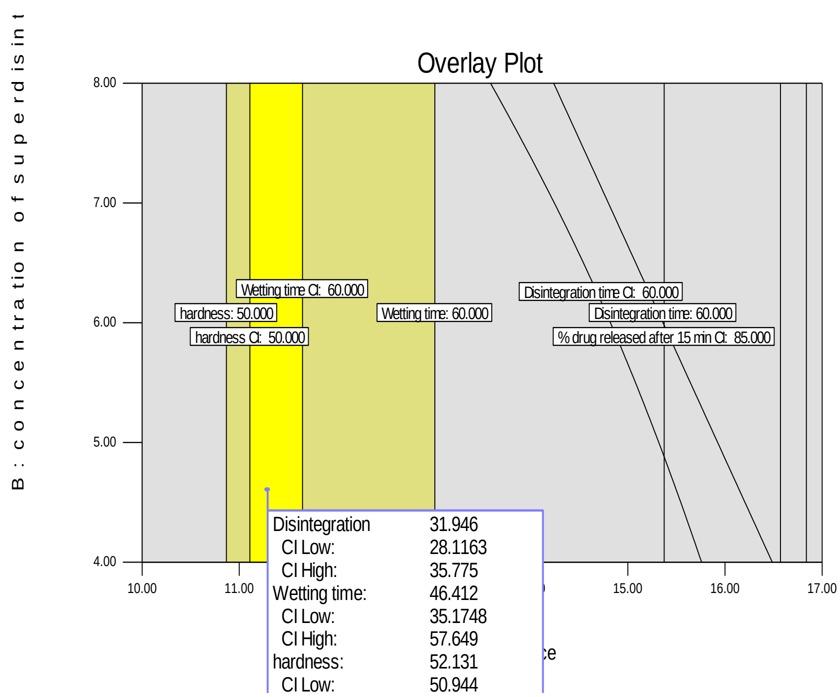


Figure3. 16: Optimum region identified by overlaying plots of the five responses as functions compression force and amount of superdisintegrant.

3.6.6.3 Confirmation test

To experimentally confirm the validity of the obtained optimal point, confirmation experiments were carried out at the optimal combinations of the factors (A= 11.29, B= 4.60, C=1.6:1 and D=1.26:1). Three batches of taste masked ODTs were prepared according to the optimized formulation. Then, disintegration time, wetting time, hardness, and % drug release after 5 and 15 min. were determined.

Table 3.18 presents the predicted values, experimental results and the percentage error values obtained at optimal levels of the factors. The values of percentage errors were below 5%, confirming that the experimental values of the optimized formulations agree well with the predicted values. The close resemblance between the observed and predicted response values indicate the robustness of the predictions, as well as the validity of the generated model.

Table 3. 18: Formulations prepared based on the predicted and the experimental values of the various responses (n = 3).

Response	Predicted value	Experimental value	% Error
Disintegration time: Y1 (sec)	31.95	33.10 ± 0.34	3.60
Wetting time: Y2 (sec)	46.41	48.32 ± 1.42	4.11
Hardness:Y3 (N)	52.13	54.30 ± 1.35	4.16
Drug release after 5 min: Y4 (%)	62.21	60.00 ± 1.76	3.55
Drug release after 15 min: Y5 (%)	90.03	88.00 ± 1.08	2.25
Friability: (%)	-	0.253 ± 0.02	-

3.7 Formulation and Characterization of the optimized taste masked phenobarbitone ODTs

Microsphere formulation containing polymer to drug ratio of 1.26:1were prepared at stirring rate of 500 rpm and characterized, Table 3.19. As shown in the table, the angle of repose, Hausner ratio and Carr's index values of the optimized microspheres have good flow property. The percentage yield and DEE and the mean particle size of microsphere were well within acceptable range.

Table 3. 19: Physicochemical characteristics of the optimized phenobarbitone microspheres and microsphere blend (mean ± SD, n=3).

Parameters	Experimental values Microsphere	Experimental values Microsphere blend
Particle size (µm)	424.10±3.50	-
Yield (%)	61.01±1.51	-
DEE (%)	92.68±1.25	-
Bulk density (g/cm3)	0.32±0.02	0.55 ± 0.02
Tapped density (g/cm3)	0.36±0.02	0.62 ± 0.03
Angle of repose (^{o})	31.68±1.26	31.83 ± 1.59
Carr's Index (%)	11.13±0.62	11.82 ± 0.44
Hauser ratio	1.13±0.01	1.13 ± 0.01
Flow rate (g/sec.)	-	4.08 ± 0.21

The microsphere blend was prepared by mixing phenobarbitone loaded microspheres with all the ingredients. Characterization of the powder blend also showed that the powder blend of the optimized formulation has a very good flow and compressibility properties (Table 3.19).

As has been discussed, the tablets have also the desired tablet characteristics, Table 3.19. Moreover, the complete release profile of the optimized formulation for all the three batches taken was determined, Fig 3.17 and were compared with a locally manufactured conventional phenobarbitone tablet. The results showed that the optimized taste masked ODTs showed superior *in-vitro* drug release profile.

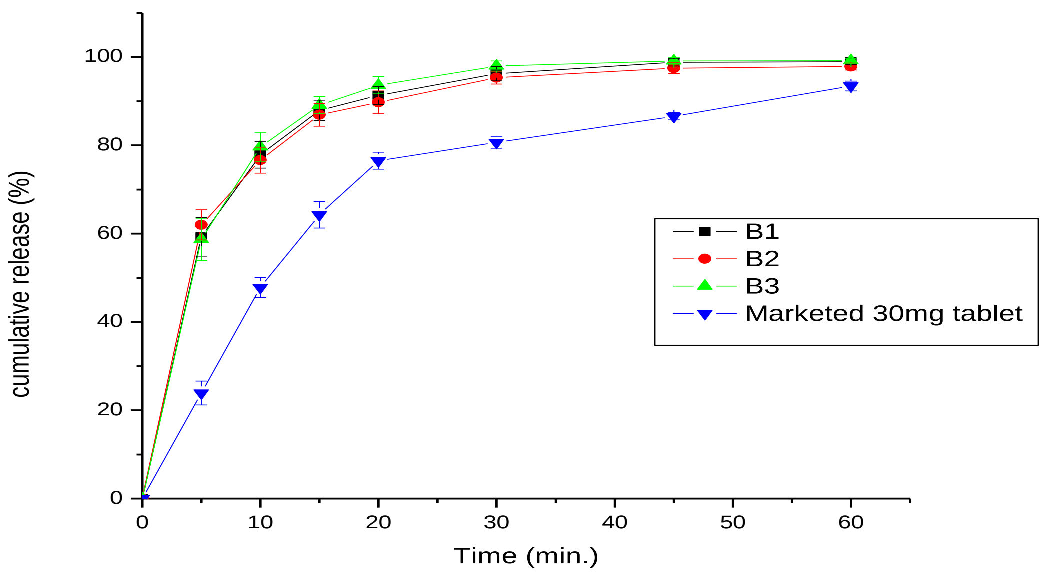


Figure 3.17: Drug release profile of the (three batch of B1, B2 and B3) optimized taste- masked ODTs compared with a locally manufactured 30 mg phenobarbitone tablet.

The uniformity of dosage units can be demonstrated by either of the two methods, weight variation test or content uniformity test. For tablets of high dose drugs (pharmaceutical products like tablets, capsules, etc., which contain 25 mg or more of an active ingredient comprising 25% or more, by weight, of the dosage form unit), weight variation test is recommended by the USP30/NF25 (2007), as an effective means of assuring uniform potency. But for tablets of low dose drugs (tablet formulations containing less than 25 mg of an active ingredient comprising less than 25% by weight of the dosage form), excipients make up the bulk of the tablets weight. Therefore, content uniformity test was performed.

According to USP30/NF25 (2007) specification, the requirements for dosage form uniformity are met if the amount of the active ingredient in each of the ten dosage units as determined from the weight variation or content uniformity method lies within the range or 85.0% to 115.0% of the labeled claim and the relative standard deviation less than or equal to 6.0%. Drug content of the ten tablets lies within the range of the label claim. The International Pharmacopoeia states that Phenobarbital tablets must contain not less than 90.0% and not more than 110.0% of the amount of Phenobarbital of the label claim (IP, 2003). The assay result for the optimized Phenobarbital tablets was 96 ± 1%, indicating that the tablets comply with the IP specification.

4. CONCLUSION

Taste-masked microspheres of phenobarbitone were prepared using Eudragit® E100 employing an emulsion solvent evaporation technique and characterized. The effects of stirring rate and polymer to drug ratio on the various physicochemical properties of the microspheres were studied and optimized. A dissolution study of the microspheres showed that the release of drug from the microspheres was retarded in pH 6.8 media and, therefore, the amount of drug released from the microspheres was found to be well beyond the bitterness threshold of phenobarbitone, which was determined *in vivo*.

Direct compression of the microsphere blend showed that both formulation variables (amount of superdisintegrant, MCC to mannitol ratio, and polymer to drug ratio) and a process variable (compression force) significantly affected the various responses, such as disintegration time, wetting time, tablet hardness, and drug release of the prepared taste-masked phenobarbitone ODTs. The RSM based on 2^{4-1} fractional factorial experimental design was employed to obtain an optimum formulation with rapid disintegration time, wetting time, acceptable hardness and enhanced dissolution properties. Accordingly, the desired optimum condition was obtained at 11.29 KN compression force, 4.60% SSG, 1.6:1 MCC to mannitol ratio and 1.26:1polymer to drug ratio. Under these conditions, the disintegration time, wetting time, hardness, % drug release within 5 min and, % drug release within 15 min were 33.10 ± 0.34 sec, 48.32 ± 1.42 sec, 54.30 ± 1.35 N, 60.00 ± 1.76% and 88.00 ± 1.08%, respectively. The observed responses were found to be in close agreement with the predicted values for the optimized formulations. Moreover, the validity of obtained optimal point was confirmed by the low magnitude of percent prediction error. FT-IR spectra of phenobarbitone and physical mixture of phenobarbiton and Eudragit® E100 suggested no interaction between the drug and polymer.

Therefore, it can be concluded that taste-masked phenobarbitone ODTs, which is suitable for pediatric, geriatric and other special population patients was successfully developed.

5. **SUGGESTIONS FOR FURTHER WORK**

- Determine the short and long term stability of the formulation; and
- Investigate the *in vivo* performance of the optimized formulation using animal models and human volunteers

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